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RESEARCH ARTICLE

New Microsatellite Markers for the Common Tern (*Sterna hirundo*) Developed with 454 Shot-Gun Pyrosequencing

Susann Janowski^{1,*}, Ina Gross¹, Hedwig Sauer-Gürth¹, Dieter Thomas Tietze¹, Markus Grohme², Marcus Frohme², Peter Becker³ and Michael Wink¹

¹Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, Im Neuenheimer Feld 364, 69120 Heidelberg, Germany

²Molecular Biotechnology and Functional Genomics, Technical University of Applied Sciences, Hochschulring 1, 15745 Wildau, Germany

³Institute of Avian Research "Vogelwarte Helgoland", An der Vogelwarte 21, 26386 Wilhelmshaven, Germany

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Abstract: Long term studies, focusing on population- and socio-biology research, require the unequivocal identification of individuals. DNA studies with Short Tandem Repeats (STR loci) became a widespread tool in population genetics. We used the next-generation sequencing (NGS) approach with 454 shot-gun pyrosequencing to identify 13 new polymorphic STR loci for the Common Tern, *Sterna hirundo*. To enlarge the marker set we added two more loci originally developed for Black-legged Kittiwake (*Rissa tridactyla*) and Red-billed Gull (*Chroicocephalus scopulinus*) and arranged these 15 loci into three multiplex PCR panels for high throughput genotyping. Loci characterization demonstrated that our marker set is of high quality. A PIC value of about 0.67 and a power of exclusion value of 0.99 were reached. Deviation from Hardy-Weinberg expectations of some loci and low frequencies for null alleles are interpreted as a result of inbreeding and founder effect in the investigated tern colony. We used a test data set of this well-studied breeding colony of Common Tern at Banter Lake, Wilhelmshaven, Germany, to perform a parentage test. Parent-chick relationships, known from the social pedigree of that colony, were compared with genetically calculated ones. In order to test our markers and the used parentage program COLONY, we conducted six competing data sets with varying completeness of included parental genotypes. By including fully sampled parent pairs of known family assignment, results were correct for nest mates, single parents and parent pairs. Our marker set provides a powerful tool to investigate life-time reproductive success and other issues of population and socio-biology for Common Terns, e.g. in the aforementioned colony monitored for decades.

Keywords: Common Tern, COLONY Software, Genotyping, Microsatellites, Multiplex PCR, Next-Generation Sequencing, Parentage Analysis, *Sterna Hirundo*.

INTRODUCTION

Population and socio-biology research of birds requires long-term studies. Monitoring of populations or breeding colonies over a long period of time depends on the unequivocal identification of individuals. Trapping and re-trapping as well as the utilization of different tagging methods (metal and color rings, pit-tags, wing-tags, radio- and satellite transmitters) are among the most common means. These methods are extremely useful also in evaluating animal movements, especially in migrating species, as well as investigating habitat use in breeding and wintering areas, for example in Montagu's harriers [1 - 4] among many others.

However, some of them are invasive and may have possible influences on the survival of individuals [5 - 7], which could for example affect interpretation of dispersal, philopatry and survival rate. Furthermore, methodical challenges may arise from different recovery probabilities.

* Address correspondence to this author at the Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, Im Neuenheimer Feld 364, 69120 Heidelberg, Germany; Tel: +49 6221 54-4847; Fax: +49 6221 54-4884; E-mails: susann.janowski@outlook.com, susann.janowski@freenet.de

DNA analyses might be an additional and sometimes more powerful tool to identify individuals. It could improve studies on philopatry and survival and enhances research topics like connectivity and exchange rates between populations as well as genetic diversity within and between (sub)populations [8]. They also possess the advantage to provide detailed knowledge of kinship and demographic structure within a particular population or breeding colony, which can be used for life-history analyses. Furthermore, utilizing parentage tests via genetic markers, already uncovered many peculiarities in breeding systems such as extra-pair parentage in many bird species [9 - 12]. Among genetic applications, microsatellites (short tandem repeats, STR loci) and SNPs (single nucleotide polymorphisms) are presently among the most useful genetic markers for population genetics [13 - 15].

With the development of next-generation sequencing (NGS) approaches, the identification of highly variable markers for non-model species became efficient and cost saving [16 - 18]. Numerous species-specific marker sets have been developed [19 - 26] in recent years.

We used NGS to identify new polymorphic STR loci for the Common Tern, *Sterna hirundo* (Linnaeus, 1758) and developed three multiplex PCR panels for high throughput genotyping. Microsatellites are among the most often used genetic markers to identify individuals for population studies such as paternity testing [9, 27 - 31] population structure [32, 33] and phylogeography [34 - 36]. The paramount advantages of codominant multi-allelic markers has been shown [23, 37].

In order to evaluate the suitability of the marker set for parentage assignments, we characterized the loci regarding common parameters such as number of alleles, observed and expected heterozygosity, deviation from Hardy-Weinberg expectations, polymorphism information content, power of exclusion and null-allele frequencies, as well as parameters concerning identity, sibship and parentage discrimination. To test the ability of parentage assignment in reality we used samples from a well-studied breeding colony of Common Tern. The investigated breeding colony is located on an artificial island on the Banter Lake in Wilhelmshaven, Germany (53°30' N, 8°06' E) [38, 39]. The widely used parentage program COLONY 2.0 [40] was used. The test data set comprised samples of a known social pedigree. Since 1992 the social pedigree of the Banter Lake tern colony has been investigated via an ingenious registration system that enables a completely monitored colony. Adults and chicks are marked with subcutaneous transponders that can be recorded automatically at the breeding sites [38]. Numerous different studies dealing with population ecology have been derived from these data [41 - 44]. By investigating this well studied colony, we were able to compare a data set of known kinships (chicks and their social parents) with genetically assigned ones. Furthermore, we wanted to learn about required quality of genotyping data and the reliability of the used parentage program COLONY. This software is able to assign missing parents even without parental genotypes. It defines parents with a symbol ('#' for mothers and '*' for fathers) and a consecutive number, which enables sibship determination. For our quality test, we conducted six different parentage tests and compared them with our known pedigree. Data sets for these tests varied in their completeness of available parental genotypes so that COLONY had to construct the pedigree with more or less complete genetic information. Sampling of genetic material is often difficult in wild animals and achieving material from complete populations is often far from being realistic. Hence, assignment results of these competing data sets may give us an idea about the required amount of parental genotypes in parentage assignment tests for future studies. Moreover it could enable researchers to interpret and evaluate likelihood-based parentage studies in general conducted with COLONY. The development of new species-specific STR markers for the Common Tern now also facilitates a long-term population genetic study of this colony, to investigate for example heterozygosity, fitness and inbreeding, among many other topics. New microsatellite markers will help to determine the population genetic structure of the Common Tern such as it has already been done for the endangered Roseate Tern, *Sterna dougallii*, in the northwestern Atlantic and western Australia [45, 46].

MATERIALS AND METHODOLOGY

Blood Sampling

DNA samples from juvenile and adult Common Terns were collected in a well-monitored colony in the harbour area of Wilhelmshaven (Banter Lake, German North Sea coast). During the breeding season, samples from non-fledged nestlings were obtained from blood of quills from body feathers plucked before fledging or from tissue samples collected from chicks found dead on the ground. Triatomine bugs, *Dipetalogaster maxima* Uhler, 1894 (Heteroptera, Reduviidae) were used to sample blood from incubating adult birds [47, 48]. Since most of the breeding pairs were equipped with a transponder, we were able to identify the social parents for each chick. Feathers, blood samples and

tissue material from dead birds were stored in an EDTA buffer (10% EDTA, 0.5% NaF, 0.5% thymol, 1%, Tris-HCL, pH = 7.5) at 4 °C until DNA extraction.

DNA Extraction, 454 Pyrosequencing and Primer Development

For DNA extraction we used a standard proteinase K (Merck, Darmstadt) protocol [49]. 454 shot-gun pyrosequencing (NGS) on a GS Junior sequencer (454 Life Sciences/Roche Applied Science) was used to create a genomic library of the Common Tern, using the GS FLX Titanium Rapid Library Preparation Kit following the manufacturer's recommendations. Starting material for the sequencing library was 500 ng genomic DNA. Emulsion PCR was carried out with a ratio of two DNA copies per bead. A single sequencing run yielded 56,755 reads with an average read length of 391 (± 165) base pairs, totaling ~22.19 Mb of sequence data. Sequence reads were scanned with MSATCOMMANDER 0.8.1 [50] for repetitive loci. In total, 233 STR motifs (excluding mononucleotide repeats) could be identified. Using Primer3 software [51] we found 96 primer pairs of which 80 were unique. Of those, 48 microsatellite loci containing at least six repeats were tested for polymorphisms. Ten Common Tern DNA samples were amplified by PCR under the following conditions: a PCR reaction of 25 μ L contained 60 ng of total genomic DNA, 0.4 pmol/ μ L of each forward and reverse primer, 0.1 mM of dGTP, dCTP and dTTP, as well as 45 μ M of dATP, 1.5 x PCR buffer (Bioron), 0.15 units of Top-Taq DNA polymerase (Bioron), 1 μ Ci [α - 33 P]-dATP (Amersham Biosciences) and a variable amount of mono distilled water to reach a volume of 25 μ L. In a TGradient ThermoCycler (Biometra) we performed the following thermocycling program: initial denaturing for 5 min at 95 °C, followed by 38 cycles of 45 s at 95 °C, 60 s at 50–58 °C, 90 s at 72 °C, followed by a final extension step at 72 °C for 10 min and a cooling step at 15 °C for storage. PCR products were denatured (95 °C for 5 min) for electrophoresis on a vertical high-resolution polyacrylamide gel (Urea 5%) at 65 W for 1.5 h (run length ca. 40 cm). An X-ray film (Hyperfilm-MP; Amersham) was placed on the dried gel for 1–2 days for autoradiography and afterwards was developed with X-ray developer and fixer (Kodak). Six primer pairs showed no amplification products, whereas the remaining 42 amplified well. Twenty-four of them produced several different allele bands and were used for further development steps, including multiplex PCRs. In order to enlarge the marker set, we included six primers developed for Black-legged Kittiwake (*Rissa tridactyla*) and Red-billed Gull (*Chroicocephalus scopulinus*) [52, 53].

Primer Labelling, Multiplex PCR and Fragment Length Analysis

Of the 24 newly developed and tested loci 11 failed to amplify properly in multiplex PCRs or showed evidence for null alleles after locus characterization and thus had to be rejected. Furthermore, we tested primers recently isolated from Roseate Terns (*Sterna dougallii*) that were shown to amplify in Common Terns [45, 46]. Unfortunately these primers did not match to our multiplex PCR panels. Instead, two primers developed for Black-legged Kittiwake and Red-billed Gull [52, 53] performed well in our test PCRs and were included in the marker set (see result part). With these resulting 15 loci, we established three multiplex PCR panels, each containing five different primer pairs. All forward primers were labeled at the 5' end with one of three different fluorescent dyes (6-FAM and HEX produced by Eurofins MWG Operon, as well as NED produced by Applied Biosystems). Primers with overlapping allele size ranges were labeled with different dyes, whereas those with non-overlapping ranges were labeled with the same dye.

We used Type-it Microsatellite PCR Kit (Qiagen) for Multiplex PCRs under the following conditions: a reaction volume of 15 μ L contained 0.09–0.24 pmol/ μ L of each forward and reverse primer, 15 ng of total DNA, 7.5 μ L of 2x Type-it Multiplex PCR Master Mix and a variable amount of RNase-free water to reach the end-volume of 15 μ L. Thermocycling started with an initial denaturing step at 95 °C for 5 min, followed by 29 cycles of 30 s at 95 °C, 90 s at 58 °C for each multiplex set and 72 °C for 30 s, a final elongation step at 60 °C for 30 min and a cooling step at 4 °C.

A 96-well plate of diluted PCR products (2.5 μ L PCR product mixed with 7.5 μ L sterile filtrated mono distilled water) was analyzed on a Applied Biosystems DNA-Analyzer (ABI 3730) by GATC, Köln, Germany. As internal size standard ET-ROX 500 (Amersham Biosciences) was added to each sample. Peak Scanner Software 2 (Applied Biosystems) was used to analyze result files, which produces decimal numbers for each fragment. We wrote a script in R v3.1.0 [54] to round the decimal numbers for each allele to full allele units in a more objective way. To first define the expected number of alleles per locus, we sorted all occurring unrounded alleles at a specific locus per size and plotted them in a diagram. Allele units were defined when significant gaps between fragment sizes became clear [17]. For each marker, rounding was performed by comparing each allele with the previous one by taking into account the locus-specific repeat-motif length and a pre-defined minimum discriminatory distance. If the distance between two subsequently compared alleles was smaller than the defined distinction distance, both alleles were rounded to the same

value. On the contrary, a larger value between two compared alleles resulted in assigning two different alleles. To achieve an accurate rounding result we also manually compared the received rounded alleles with the sizes gained from Peak Scanner Software 2.

STR Characterization and Parentage Assessments

For STR characterization we used 46 adult Common Tern samples. Parentage assignment was conducted with these parents and 85 of their chicks. Samples were collected in the breeding seasons of 2000–2012 and corresponded to 23 different families; they included both parents and their offspring that were produced in the study period. The number of chicks per family varied between a single one and 11 individuals. 56.5% of families produced at least three chicks. All of the analyzed samples were fully genotyped, which means that all alleles of the 15 markers were determined.

For single locus characterization we used CERVUS 3.0 [55] to estimate number of alleles (N_a), observed and expected heterozygosity values (H_{obs} and H_{exp}) as well as polymorphism information content (PIC). Moreover, the program was used to estimate PIC, non-exclusion probabilities for parents, individual identity and sib identity probabilities for the whole marker set. Genepop V4 [56] was used to calculate exact values for Hardy-Weinberg expectations (HWE) with a Bonferroni correction for multiple comparisons (probability test: dememorization steps: 100000, batches: 500, iterations per batch: 10000) and to estimate null allele frequencies. PowerStats V12 (Promega Corporation 2000) was used to determine power of exclusion values (PE) for each locus and across all loci. COLONY 2.0 [40] was chosen for parentage assignment. We did not add information about full-sib relationships for computation, which is facultative in the program’s pre-setting. A polygamous mating system was assumed for both sexes in the calculation, since samples from half-sibs of different years were present in the data set. To test our markers and COLONY software for assignment quality we prepared six contrasting data sets. While genotypes for nestlings remained complete in all of the six calculations, entire used parental genotypes varied: Calculation one (23M.23F) contained all available parental genotypes, the second one (11M.23F) contained 100% of paternal genotypes but only 11 randomly selected ones for mothers. The third one (11F.23M) contained 100% of maternal genotypes but only 11 randomly selected ones for fathers. Calculation four (0M.23F) contained only paternal genotypes while data set 0F.23M only maternal ones and data set 0M.0F no parental genotypes at all. Assignment results concerning correct allocation of nest mates and parent pairs were compared with information from the social pedigree. A family was defined according to chicks that were sampled in the same nest and their social parents that were identified through transponder readings. Additionally to COLONY software we confirmed the parentage assignments by eye and checked for sex-linked loci. This was a necessary controlling step regarding the reliability of the new marker set.

RESULTS

By means of next-generation sequencing 13 new polymorphic STR loci could be isolated for the Common Tern. We added two markers (locus K32, isolated from Black-legged Kittiwake [53] and locus RBG18, isolated from Red-billed Gull [52]) and established three different multiplex panels, containing together 15 loci (Table 1).

Table 1. Three multiplex PCR panels for the amplification of microsatellite loci in the Common Tern.

Multiplex Panels	Locus	Primer Sequence (5’–3’)	Dye	Concentration [pmol/μL]
Panel 1	K32	F: CATTGCACGAGTGTTAAGCTG	FAM	0.12
		R: AAGGGTGCCTGTCCTTGTC		
	MsSh22	F: GCCAGCTCTGCAATTCTACG	HEX	0.24
		R: ACCCAGTAACCAGGCAGATG		
	RBG18	F: AAAGGGCTGCTCATAGTACG	HEX	0.09
		R: GTAGCATCATGTCTTCCCGC		
	MsSh07	F: AGGCATTCTGAAATAGTGGGC	NED	0.09
		R: AGGTCAGATATGTTTCGTTGTGG		
	MsSh03	F: GTTGGTGCTTGGGAAGTAGGC	NED	0.20
		R: AATGCCTGTCAAGTTGGGTGG		
Panel 2	MsSh23	F: GCGCATGAATGAGAGACAATTG	FAM	0.11
		R: TGTATCCATGGCTCAGCTACC		
	MsSh18	F: AGAGCCAGGCACCCATATTC	FAM	0.23

(Table 3) contd....

Multiplex Panels	Locus	Primer Sequence (5'-3')	Dye	Concentration [pmol/μL]
		R: CAGGTGGGTCGTCATGAGAG		
	MsSh20	F: GTCCGTACAGAGGTTAACGC	HEX	0.12
		R: ATGGTCACACTCGTCTCACC		
	MsSh21	F: TGGTGTGCATGCATTGTGG	NED	0.13
		R: GGCATTAGTCAGGGTGTGTC		
	MsSh31	F: AGAGAGAAGGAATTGGTGTAGC	NED	0.09
		R: GGACCTGAGATCACCACAAG		
Panel 3	MsSh10	F: GCTGCGTATGTCCCAACTTG	FAM	0.12
		R: CCAAATTGCTCCTCTGGCTG		
	MsSh08	F: GTAGCCTCAGTACTGGTGGG	FAM	0.11
		R: CCGTCTAAAGCCAGCACTTG		
	MsSh09	F: GTGTCACCTGCCAGAAATG	HEX	0.16
		R: AGGGCATACATCCCTCATCC		
	MsSh48	F: ATACACTGGCGGATGTTGTC	HEX	0.19
		R: ACAAGATGACACAGAGCTGC		
	MsSh37	F: AGCCAAACGACCTGAGTGAG	NED	0.14
		R: GGAGAAGAGATGGTGTGTGC		

Each forward primer is labeled with one of three different fluorescent dyes.

In Table 2 we characterized each locus. Mean number of alleles per locus is 8 and a calculation of PIC across all loci revealed a value of 0.67. Only three loci (MsSh10, MsSh37 and K32) showed PIC values below 0.50. Significant deviation from Hardy-Weinberg expectations was found for MsSh18 and MsSh23. Furthermore, null alleles may exist in locus MsSh07, MsSh20, MsSh21, MsSh23 and MsSh31, when setting the acceptance boundary to 0.05. None of the loci appeared to be sex-linked. Sex-linked loci would result in accumulated homozygote alleles in one sex, while the other one mainly would appear heterozygote.

Table 2. Characterization of the 15 STR loci for the Common Tern.

Locus	Repeat	N _a	Size Range [bp]	H _{obs}	H _{exp}	HWE	PIC	PE	Null
MsSh03	(AAAC) ₆	5	292–308	0.63	0.62	0.90	0.57	0.33	0.00
MsSh07	(ATTC) ₇	6	192–216	0.54	0.65	0.06	0.59	0.23	0.10
MsSh08	(ATTC) ₇	8	213–253	0.80	0.81	0.89	0.77	0.61	0.02
MsSh09	(ATCC) ₁₀	7	237–261	0.87	0.84	0.67	0.81	0.73	0.00
MsSh10	(AGGC) ₇	4	147–163	0.57	0.54	0.88	0.48	0.25	0.00
MsSh18	(AAT) ₁₇	12	221–284	0.80	0.82	0.03	0.79	0.61	0.01
MsSh20	(AC) ₁₂	12	102–132	0.74	0.87	0.07	0.85	0.49	0.06
MsSh21	(AC) ₁₀	8	113–135	0.80	0.81	0.08	0.78	0.61	0.11
MsSh22	(AC) ₁₅	12	131–163	0.78	0.81	0.23	0.78	0.57	0.05
MsSh23	(AC) ₁₃	10	165–197	0.63	0.78	0.03	0.74	0.33	0.10
MsSh31	(AG) ₁₀	4	171–177	0.59	0.66	0.34	0.59	0.28	0.08
MsSh37	(AC) ₉	8	392–418	0.46	0.45	0.81	0.42	0.15	0.00
MsSh48	(AAT) ₈	10	326–353	0.89	0.88	0.40	0.86	0.78	0.02
K32	(GA) ₂ (GT) ₁₂	4	109–115	0.41	0.38	0.70	0.35	0.12	0.00
RBG18	(GT) ₁₁	8	173–193	0.78	0.76	0.64	0.71	0.57	0.00

N_a: number of alleles; H_{obs}: observed heterozygosity; H_{exp}: expected heterozygosity; HWE: deviation from Hardy-Weinberg expectations; PIC: Polymorphism information content; PE: power of exclusion; Null: null allele frequency.

Parentage Analysis

Different statistics for parentage analyses and identity tests are represented in Table 3. A power of exclusion (PE) value over all loci of about 0.99 was reached. The inclusion of parental genotypes in kinship calculation leads to a much higher probability of excluding an unrelated candidate parent from parentage of an arbitrary offspring than without including parental genotypes. We assume that individual identities (NE-I) and full-sibling relationships (NE-SI) are highly reliable.

Table 3. Statistics for parentage analyses across all 15 loci.

Statistic of Interest	Probability
PEall	0.99
NE-1p	1.13*10 ⁻³
NE-2p	9.53*10 ⁻⁶
NE-pp	2.96*10 ⁻⁹
NE-I	1.74*10 ⁻¹⁵
NE-SI	2.46*10 ⁻⁶

PE_{all}: power of exclusion over all loci. Probability of combined non-exclusion of two potential parents with unknown (NE-1p) and known (NE-2p) parental genotypes, as well as for parent pairs (NE-pp). Furthermore the probability of failing distinction between two randomly selected individuals (NE-I) or full-siblings (NE-SI).

We analyzed parentage of 85 juvenile Common Terns and corresponding 46 parents (23 females and 23 males). In order to test the program as well as the suitability of our marker set, six assignment runs were conducted. Results are shown in Table 4, where each run differs by the inclusion of involved parental genotypes. For the case of providing all maternal and paternal genotypes (23M, 23F), COLONY 2.0 identified 23 different parent-chick families with their expected sibship and parent-pair combinations. By omitting 12 maternal genotypes (11M, 23F) maternal and parent pair assignment became incorrect for 1.2% of all chicks, while nest mates and fathers were correctly assigned to 100%. The erroneous assignment corresponds to one nest with only one chick. For the case of omitting 12 paternal genotypes from calculation (11F, 23M), only maternal genotypes could be correctly assigned for 100% of the cases. In two nests with two chicks the assignments failed. When supplying only genotypes of a single parental sex (0M, 23F and 0F, 23M), assignment was accurate for the supplied one but incorrect for the other sex and parent-pair combinations in 3.5% of all chicks. In total, 91.3% of nest mates were assorted correctly in both cases. In the first calculation, assignment was incorrect for one nest with one chick, one nest with two chicks and one nest with three chicks. For the second calculation mistakes were found in two nests with two chicks. Finally, assignment results became more prone to errors when no parental genotypes were provided at all. Additionally to the use of COLONY, we confirmed the parentage assignments by eye. Mismatches among genotypes of expected parents were not present.

Table 4. Comparison of parentage assignment for six calculations.

Assignment result for:	Included parental genotypes					
	23M, 23F	11M, 23F	11F, 23M	0M, 23F	0F, 23M	0M, 0F
correct nest mates	100.0	100.0	91.3	91.3	91.3	60.9
correct father	100.0	100.0	96.5	100.0	96.5	87.1
correct mother	100.0	98.8	100.0	96.5	100.0	88.2
correct parent pair	100.0	98.8	96.5	96.5	96.5	78.8

Parentage was calculated for 85 chicks, 23 mothers and 23 fathers. Assortment of nest mates, single fathers, mothers and parent pairs to available chicks were tested depending on provided parental genotypes. 23M, 23F: all parental genotypes are provided; 11M, 23F: 12 maternal genotypes are left out from calculation; 11F, 23M: 12 paternal genotypes are left out from calculation; 0M, 23F and 0F, 23M respectively: only maternal or paternal genotypes were involved; 0M, 0F: parentage test was performed without parental genotypes.

DISCUSSION AND CONCLUSION

Using 454 pyrosequencing we identified 13 new loci that provide a useful tool for genetic analyses and parentage tests of Common Terns. Only three loci showed PIC values less than 0.5, which implies a moderate polymorphic-information content for them. Nevertheless, the whole marker set is highly informative, due to an overall high PIC value and high discrimination probabilities for parents and sibs. Genepop software revealed deviation from Hardy-Weinberg expectations for two loci and a slight to moderate possibility of existing null alleles for five loci. We interpret these findings as a result of the small initial colony size. The colony originated from a small founder population so that inbreeding is possible [57]. Nevertheless, the present marker set is of very good quality, since identification of individuals, siblings and parent-pairs is given. This is evidenced by our comparison of expected (social) and assigned (genetic) kinships, when varying the amount of included parental genotypes. The comparison of our six test data sets shows that, by providing genetic information of all possible parents, kinship can be resolved to a very high degree and resamples the expected pedigree. Without any parental genotypes, assignment result for nest mates, single parents as well as for parent pairs is less reliable, compared to the known pedigree. This finding is not surprising, since relatedness is normally much higher in small colonies (such as the investigated one) than in large populations. A moderate

sampling success of all possible parents where at least one parent of a pair is available or one sex could be sampled completely is the most realistic scenario for many population studies. Under this condition, we achieved in our test data sets a value of 96.5–100% correctness for single and parent pairs. In that way, we conclude that our new marker set is able to discriminate adequately between closely related individuals under realistic sampling conditions. Therefore, we recommend the following points to enable correct assignment results in wild populations and without known pedigree information: Development of species specific, high quality markers, utilization of fully genotyped samples without missing alleles, supplemented as much as possible by corresponding parental genotypes. Furthermore, families having at least three chicks and at least one parental genotype very likely produce unequivocal identifications. Adhering to such a strict data selection the COLONY software should be able to assign the correct missing parent in most cases. Hence, correct full- and half-sibship determination will become possible. Since in most biological studies, availability of parental genotypes is the most crucial point and samples of parent pairs are often incomplete, COLONY software is a powerful program to identify the missing parent.

The three multiplex STR panels will provide a cost and time saving approach to genotype large numbers of individuals of the Common Tern. They will supplement and expand the research on the social pedigree of the breeding colony on the Banter Lake. Our aim is to genotype thousands of samples in a long-term population study. Together with the automated transponder based identification system, it will enable us to receive a more detailed insight into breeding system, life-time reproductive success, kinship and family structures within this colony.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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REFERENCES

- [1] Liminana R, Garcia JT, Gonzalez JM, *et al.* Philopatry and natal dispersal of Montagu's harriers (*Circus pygargus*) breeding in Spain: a review of existing data. *Eur J Wildl Res* 2012; 58: 549-55. [<http://dx.doi.org/10.1007/s10344-011-0602-2>]
- [2] Liminana R, Soutullo A, Urios V. Autumn migration of Montagu's harriers *Circus pygargus* tracked by satellite telemetry. *J Ornithol* 2007; 148: 517-23. [<http://dx.doi.org/10.1007/s10336-007-0182-9>]
- [3] Trierweiler C, Klaassen RH, Drent RH, *et al.* Migratory connectivity and population-specific migration routes in a long-distance migratory bird. *Proc R Soc Biol Sci Ser B*. 281. [<http://dx.doi.org/10.1098/rspb.2013.2897>]
- [4] Trierweiler C, Mullié WC, Drent RH, *et al.* A Palaearctic migratory raptor species tracks shifting prey availability within its wintering range in the Sahel. *J Anim Ecol* 2013; 82(1): 107-20. [<http://dx.doi.org/10.1111/j.1365-2656.2012.02036.x>] [PMID: 23137184]
- [5] Authier M, Peron C, Mante A, Vidal P, Gremillet D. Designing observational biologging studies to assess the causal effect of instrumentation. *Methods Ecol Evol* 2013; 4: 802-10. [<http://dx.doi.org/10.1111/2041-210X.12075>]
- [6] Trefry SA, Diamond AW, Jesson LK. Wing marker woes: a case study and meta-analysis of the impacts of wing and patagial tags. *J Ornithol* 2013; 154: 1-11. [<http://dx.doi.org/10.1007/s10336-012-0862-y>]
- [7] Dann P, Sidhu LA, Jessop R, *et al.* Effects of flipper bands and injected transponders on the survival of adult Little Penguins *Eudyptula minor*. *Ibis* 2014; 156: 73-83. [<http://dx.doi.org/10.1111/ibi.12122>]
- [8] Vassart M. New tools in conservation genetics. *Rev Med Vet (Toulouse)* 1995; 146: 171-80.
- [9] Briggs CW, Collopy MW. Extra-pair paternity in Swainson's Hawks. *J Field Ornithol* 2012; 83: 41-6.

- [http://dx.doi.org/10.1111/j.1557-9263.2011.00354.x]
- [10] Fridolfsson A-K, Gyllensten UB, Jakobsson S. Microsatellite markers for paternity testing in the Willow Warbler *Phylloscopus trochilus*: high frequency of extra-pair young in an island population. *Hereditas* 1997; 126: 127-32. [http://dx.doi.org/10.1111/j.1601-5223.1997.00127.x]
- [11] Martínez JJ, de Aranzamendi MC, Masello JF, Bucher EH. Genetic evidence of extra-pair paternity and intraspecific brood parasitism in the monk parakeet. *Front Zool* 2013; 10(1): 68. [http://dx.doi.org/10.1186/1742-9994-10-68] [PMID: 24209709]
- [12] Schmoll T, Winkel W, Lubjuhn T. Molecular genetic evidence for mixed maternity in broods of the Coal Tit *Parus ater*. *Vogelwarte* 2008; 46: 223-7.
- [13] Sunnucks P. Efficient genetic markers for population biology. *Trends Ecol Evol (Amst)* 2000; 15(5): 199-203. [http://dx.doi.org/10.1016/S0169-5347(00)01825-5] [PMID: 10782134]
- [14] Jarne P, Lagoda PJ. Microsatellites, from molecules to populations and back. *Trends Ecol Evol (Amst)* 1996; 11(10): 424-9. [http://dx.doi.org/10.1016/0169-5347(96)10049-5] [PMID: 21237902]
- [15] Herráeza DL, Schäfer H, Mosner J, Fries H-R, Wink M. Comparison of microsatellite and single nucleotide polymorphism markers for the genetic analysis of a Galloway cattle population. *Z Naturforsch, C, J Biosci* 2005; 60(7-8): 637-43. [PMID: 16163842]
- [16] Gardner MG, Fitch AJ, Bertozzi T, Lowe AJ. Rise of the machines: recommendations for ecologists when using next generation sequencing for microsatellite development. *Mol Ecol Resour* 2011; 11(6): 1093-101. [http://dx.doi.org/10.1111/j.1755-0998.2011.03037.x] [PMID: 21679314]
- [17] Guichoux E, Lagache L, Wagner S, *et al.* Current trends in microsatellite genotyping. *Mol Ecol Resour* 2011; 11(4): 591-611. [http://dx.doi.org/10.1111/j.1755-0998.2011.03014.x] [PMID: 21565126]
- [18] Santana Q, Coetzee M, Steenkamp E, *et al.* Microsatellite discovery by deep sequencing of enriched genomic libraries. *Biotechniques* 2009; 46(3): 217-23. [http://dx.doi.org/10.2144/000113085] [PMID: 19317665]
- [19] Brennan PL, Hyseni C. Development of microsatellite markers for parentage analysis in the great tinamou (*Tinamus major*). *Mol Ecol Resour* 2008; 8(4): 933-5. [http://dx.doi.org/10.1111/j.1755-0998.2008.02122.x] [PMID: 21585934]
- [20] Busch JD, Katzner TE, Bragin E, Keims P. Tetranucleotide microsatellites for *Aquila* and *Haliaeetus* eagles. *Mol Ecol Notes* 2005; 5: 29-41. [http://dx.doi.org/10.1111/j.1471-8286.2004.00823.x]
- [21] Ferreira JM, Burnham-Curtis M, Schunck F, *et al.* Development of novel polymorphic microsatellite markers for four bird species exploited by the illegal wildlife trade in Brazil. *Conserv Genet Resour* 2015; 7: 435-6. [http://dx.doi.org/10.1007/s12686-014-0389-z]
- [22] King AG, Cooke GM, Johnson RN, Major RE. Development and multiplex genotyping of eighteen novel microsatellite markers for a threatened saltmarsh bird *Epthianura albifrons* (Meliphagidae). *Conserv Genet Resour* 2012; 4: 893-6. [http://dx.doi.org/10.1007/s12686-012-9667-9]
- [23] Martínez-Cruz B, David VA, Godoy JA, *et al.* Eighteen polymorphic microsatellite markers for the highly endangered Spanish imperial eagle (*Aquila adalberti*) and related species. *Mol Ecol Notes* 2002; 2: 323-6. [http://dx.doi.org/10.1046/j.1471-8286.2002.00231.x]
- [24] Steeves TE, Hale ML, Gemmill NJ. Development of polymorphic microsatellite markers for the New Zealand black stilt (*Himantopus novaezelandiae*) and cross-amplification in the pied stilt (*Himantopus himantopus leucocephalus*). *Mol Ecol Resour* 2008; 8(5): 1105-7. [http://dx.doi.org/10.1111/j.1755-0998.2008.02170.x] [PMID: 21585985]
- [25] Tingay RE, Dawson D, Pandhal J, *et al.* Isolation of 22 new *Haliaeetus* microsatellite loci and their characterization in the critically endangered Madagascar fish-eagle (*Haliaeetus vociferoides*) and three other *Haliaeetus* eagle species. *Mol Ecol Notes* 2007; 7: 711-5. [http://dx.doi.org/10.1111/j.1471-8286.2007.01690.x]
- [26] Topinka JR, May B. Development of polymorphic microsatellite loci in the northern goshawk (*Accipiter gentilis*) and cross-amplification in other raptor species. *Conserv Genet* 2004; 5: 861-4. [http://dx.doi.org/10.1007/s10592-004-1973-7]
- [27] Babb PL, McIntosh AM, Fernandez-Duque E, Di Fiore A, Schurr TG. An optimized microsatellite genotyping strategy for assessing genetic identity and kinship in Azaras owl monkeys (*Aotus azarai*). *Folia Primatol (Basel)* 2011; 82(2): 107-17. [http://dx.doi.org/10.1159/000330564] [PMID: 21912137]
- [28] Durrant KL, Hughes JM. Differing rates of extra-group paternity between two populations of the Australian magpie (*Gymnorhina tibicen*). *Behav Ecol Sociobiol* 2005; 57: 536-45. [http://dx.doi.org/10.1007/s00265-004-0883-5]
- [29] Overeem KR, Gabriel PO, Zirpoli JA, Black JM. Steller sex: infidelity and sexual selection in a social Corvid (*Cyanocitta stelleri*). *PLoS One* 2014; 9(8): e105257. [http://dx.doi.org/10.1371/journal.pone.0105257] [PMID: 25148039]

- [30] Sakaoka K, Suzuki I, Kasugai N, Fukumoto Y. Paternity testing using microsatellite DNA markers in captive Adélie penguins (*Pygoscelis adeliae*). *Zoo Biol* 2014; 33(5): 463-70. [PMID: 25157452]
- [31] Seddon N, Amos W, Adcock G, et al. Mating system, philopatry and patterns of kinship in the cooperatively breeding subdesert mesite *Monias benschi*. *Mol Ecol* 2005; 14(11): 3573-83. [http://dx.doi.org/10.1111/j.1365-294X.2005.02675.x] [PMID: 16156824]
- [32] Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics* 2000; 155(2): 945-59. [PMID: 10835412]
- [33] Stumpf KJ, Theimer TC, Mcleod MA, Koronkiewicz TJ. Disparity in population structuring of Southwestern Willow Flycatchers based on geographic distance, movement patterns, and genetic analyses. *J Exp Zool A Ecol Genet Physiol* 2014; 321: 577-85.
- [34] Albayrak T, Gonzalez J, Drovetski SV, Wink M. Phylogeography and population structure of Kruper's Nuthatch *Sitta krueperi* from Turkey based on microsatellites and mitochondrial DNA. *J Ornithol* 2012; 153: 405-11. [http://dx.doi.org/10.1007/s10336-011-0756-4]
- [35] Barrientos R, Kvist L, Barbosa A, et al. Refugia, colonization and diversification of an arid-adapted bird: coincident patterns between genetic data and ecological niche modelling. *Mol Ecol* 2014; 23(2): 390-407. [http://dx.doi.org/10.1111/mec.12588] [PMID: 24215522]
- [36] Song G, Yu LJ, Gao B, et al. Gene flow maintains genetic diversity and colonization potential in recently range-expanded populations of an Oriental bird, the Light-vented Bulbul (*Pycnonotus sinensis*, Aves: Pycnonotidae). *Divers Distrib* 2013; 19: 1248-62. [http://dx.doi.org/10.1111/ddi.12067]
- [37] Gerber S, Mariette S, Streiff R, Bodènès C, Kremer A. Comparison of microsatellites and amplified fragment length polymorphism markers for parentage analysis. *Mol Ecol* 2000; 9(8): 1037-48. [http://dx.doi.org/10.1046/j.1365-294x.2000.00961.x] [PMID: 10964223]
- [38] Becker PH, Wendeln H. A new application for transponders in population ecology of the Common Tern. *Condor* 1997; 99: 534-8. [http://dx.doi.org/10.2307/1369963]
- [39] Becker PH, Wendeln H, Gonzalez-Solis J. Population dynamics, recruitment, individual quality and reproductive strategies in Common Terns *Sterna hirundo* marked with transponders. *Ardea* 2001; 89: 241-52.
- [40] Jones OR, Wang J. COLONY: a program for parentage and sibship inference from multilocus genotype data. *Mol Ecol Resour* 2010; 10(3): 551-5. [http://dx.doi.org/10.1111/j.1755-0998.2009.02787.x] [PMID: 21565056]
- [41] Becker PH, Zhang H. Renesting of Common Terns *Sterna hirundo* in the life history perspective. *J Ornithol* 2011; 152: 213-25. [http://dx.doi.org/10.1007/s10336-010-0639-0]
- [42] Bouwhuis S, Vedder O, Becker PH. Sex-specific pathways of parental age effects on offspring lifetime reproductive success in a long-lived seabird. *Evolution* 2015; 69(7): 1760-71. [http://dx.doi.org/10.1111/evo.12692] [PMID: 26095174]
- [43] Gonzalez-Solis J, Wendeln H, Becker PH. Within and between season nest-site and mate fidelity in Common Terns (*Sterna hirundo*). *J Ornithol* 1999; 140: 491-8. [http://dx.doi.org/10.1007/BF01650993]
- [44] Wendeln H, Becker PH. Effects of parental quality and effort on the reproduction of Common Terns. *J Anim Ecol* 1999; 68: 205-14. [http://dx.doi.org/10.1046/j.1365-2656.1999.00276.x]
- [45] Szczyp P, Hughes CR, Kesseli RV. Novel microsatellite markers used to determine the population genetic structure of the endangered Roseate Tern, *Sterna dougallii*, in Northwest Atlantic and Western Australia. *Conserv Genet* 2005; 6: 461-6. [http://dx.doi.org/10.1007/s10592-004-4975-6]
- [46] Szczyp P, Nisbet IC, Wingate DB. Conservation genetics of the Common Tern (*Sterna hirundo*) in the North Atlantic region; implications for the critically endangered population at Bermuda. *Conserv Genet* 2012; 13: 1039-43. [http://dx.doi.org/10.1007/s10592-012-0351-0]
- [47] Arnold JM, Oswald SA, Voigt CC, et al. Taking the stress out of blood collection: comparison of field blood-sampling techniques for analysis of baseline corticosterone. *J Avian Biol* 2008; 39: 588-92. [http://dx.doi.org/10.1111/j.0908-8857.2008.04265.x]
- [48] Becker PH, Voigt CC, Arnold JM, Nagel R. A non-invasive technique to bleed incubating birds without trapping: a blood-sucking bug in a hollow egg. *J Ornithol* 2006; 147: 115-8. [http://dx.doi.org/10.1007/s10336-005-0027-3]
- [49] Sambrook J, Fritsch EF, Maniatis T. *Molecular cloning: a laboratory manual*. New York: Cold Spring Harbor Laboratory Press 1989.
- [50] Faircloth BC. msatcommander: detection of microsatellite repeat arrays and automated, locus-specific primer design. *Mol Ecol Resour* 2008; 8(1): 92-4. [http://dx.doi.org/10.1111/j.1471-8286.2007.01884.x] [PMID: 21585724]
- [51] Rozen S, Skaletsky H. Primer3 on the WWW for general users and for biologist programmers. *Methods Mol Biol* 2000; 132: 365-86.

[PMID: 10547847]

- [52] Given AD, Mills JA, Baker AJ. Isolation of polymorphic microsatellite loci from the Red-billed Gull (*Larus novaehollandiae scopulinus*) and amplification in related species. *Mol Ecol Notes* 2002; 2: 416-8.
[<http://dx.doi.org/10.1046/j.1471-8286.2002.00261.x>]
- [53] Tirard C, Helfenstein F, Danchin E. Polymorphic microsatellites in the Black-legged Kittiwake *Rissa tridactyla*. *Mol Ecol Notes* 2002; 2: 431-3.
[<http://dx.doi.org/10.1046/j.1471-8286.2002.00258.x>]
- [54] Team R Core. A language and environment for statistical computing. R Foundation for Statistical Computing 2014, Vienna, Austria, 2014. Available from: <http://www.R-project.org/>
- [55] Kalinowski ST, Taper ML, Marshall TC. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol Ecol* 2007; 16(5): 1099-106.
[<http://dx.doi.org/10.1111/j.1365-294X.2007.03089.x>] [PMID: 17305863]
- [56] Rousset F. genepop007: a complete re-implementation of the genepop software for Windows and Linux. *Mol Ecol Resour* 2008; 8(1): 103-6.
[<http://dx.doi.org/10.1111/j.1471-8286.2007.01931.x>] [PMID: 21585727]
- [57] Ludwig S, Becker PH. Immigration prevents inbreeding in a growing colony of a long-lived and philopatric seabird. *Ibis* 2012; 154: 74-84.
[<http://dx.doi.org/10.1111/j.1474-919X.2011.01199.x>]

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