

Parentage determination in kin-structured populations: microsatellite analyses in the Siberian jay *Perisoreus infaustus* during a 25-year population study

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We used nine polymorphic microsatellite markers for parentage determination in the Siberian jay *Perisoreus infaustus*, and performed analyses on 419 individuals, using feather or blood samples collected during a 25-year population study in western Finland. In strongly kin-structured populations, as in the Siberian jay, parentage determination is difficult because putative parents might be closely related and hence genetically similar. In order to increase the power of the parentage determination system we developed a new method, in which juveniles were tested against observed parent pairs rather than each parent separately. This method was based on the assumption of genetic monogamy in the study population, which was supported by several lines of evidence. The power of the parentage determination system was examined by extensive calculations of within-population and between-population mismatch frequencies. Parentage was examined for 298 juveniles, most of which were sampled within small groups of jays in the autumn. Altogether, we identified the parents of 89 % of all the juveniles sampled during the study period; 19 % of them had left their natal territory before sampling. The study shows that a priori information on the identity of the mother, or information on parental pair-bonds, may be crucial for successful parentage determination in kin-structured populations.

Key words: Parentage determination, Siberian jay, *Perisoreus infaustus*, microsatellite analyses, kin-structured populations.

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When microsatellites were introduced as a tool for parentage determination, they were considered to be the ideal markers for this purpose (Queller et al. 1993). Their main advantages are their large numbers in the genome, high levels of polymorphism, and the fact that they can be scored locus by locus with PCR. Analyses can thus be performed on tiny and even partially degraded DNA samples. Unfortunately microsa-

tellites must be cloned separately for every studied species.

Later studies using microsatellites for parentage determination have revealed new weaknesses. If the number of candidate parents is large (Coltman et al. 1998) or the candidate parents are mutually close relatives (Double et al. 1997), the efficiency of microsatellite analysis may be much weaker than initially calculated

on theoretical grounds. Furthermore, if the studied population is incompletely sampled or the allele data contain typing errors, the reliability of parentage determinations suffers (Marshall et al. 1998) and the power of analysis may be insufficient to make any conclusions at all about parentage (Taylor et al. 1997). Two basic problems may occur: (1) more than one female or male may be identified as a potential parent, or (2) no matching parent may be found. Success in parentage determinations is thus not only dependent on the exclusion power of the microsatellite loci used, but also on the mating system and population structure of the studied species, and on the proportion of individuals sampled for DNA.

In this study we investigate the problems of parentage determination in the Siberian jay *Perisoreus infaustus*, a species that lives in small territorial groups around an apparently monogamous adult pair (Ekman et al. 1994). The Siberian jay is strongly philopatric, whereby candidate parents are often closely related. We applied a set of nine microsatellite markers on a data set of feathers or blood samples collected during a long-term population study in Finland. Because of the strong kin-structure of the population we developed a new method, in which juveniles were tested against observed parent pairs rather than each parent separately. This method is based on the assumption of total monogamy in the population, and this assumption was tested in several ways. The aim of this paper is (1) to confirm the assumption of total monogamy in the study species, and thereby validate our new method for parentage assignment; (2) to check parentage within observed groups of jays; and (3) to trace the origin of juveniles that had left their natal territory before sampling. This important information will be used in later studies of the family structure and dispersal behaviour of this social bird species.

Materials and methods

Species and study area

The Siberian jay (also called ‘jay’ in this paper) is a long-lived (<20 years), resident bird species living in mature coniferous forests of the Eurasian taiga (Helle & Lillandt 1997). The jays form life-long, monogamous pair-bonds and live in permanent territories. Di-

vorces are rare, but widowed birds can establish a new territory and pair-bond. Established pairs are commonly accompanied by retained offspring and non-offspring juveniles, forming small flocks of 3–5 individuals. Juveniles may disperse at any time of the year and dispersal distances are mostly short, especially among males, and thus neighbouring males are often close relatives (pers. obs.). However, long-distance dispersal does occur, as confirmed by ringing recoveries.

This study was conducted from 1974 to 1998 in the forests around Kristinestad and Närpes in western Finland (62° 22'N, 21° 30'E), close to the Gulf of Bothnia. Jays were monitored mainly in three neighbouring forest areas (120, 70 and 155 km², respectively), separated from each other by 100–1500 m wide agricultural fields or peatlands. The study started in the first mentioned area in 1974, and was successively extended into the neighbouring areas from 1985 to 1992. The areas maintained jay populations of 7–17, 3–5 and 15–32 jay territories, respectively. These areas are referred to as different ‘populations’ in this paper. More details about the study area will be given elsewhere.

DNA sampling and data collection

DNA samples were collected during annual monitoring and capture of the birds, which was performed in summer and autumn (July–October) when food-hoarding jays could easily be attracted to feeding stations put in trees in their territories. Juvenile birds were distinguished from adults by the shape of their outermost tail-feathers (Svensson 1992). With the original intention of checking their age later, one tail-feather (generally the left outermost) was collected from most individuals since 1976. These feathers made the extensive genetic analyses possible, spanning the whole 25-year study period. During 1997–98 a 25–50 µl blood sample was taken from every captured individual (n = 158). Altogether, we have DNA samples from 419 of the total number of 542 jays ringed in this study. Of these 419 sampled birds 298 were observed as juveniles, and a large number of them were later found as adults. Feather samples were not collected from every individual during the years 1974–75 and 1989–91, and were not taken from nestlings. However, DNA samples were later collected from 28 individuals that had been ringed previously as nestlings. Feather samples were stored in paper envelopes at room temperature, while blood sam-

ples were stored frozen in 500 μ l SET-buffer (0.15 M NaCl, 0.05 M Tris, 0.001 M EDTA, pH 8.0).

All birds were ringed with unique combinations of colour rings. They were sexed by morphological measurements (males are slightly bigger), and sex determination based on morphology has been found to be almost completely correct in adult birds when checked against sexing by molecular techniques (unpublished data). Our goal was to check every individual bird at each feeding station annually during the years 1974–98. To make sure that every surviving individual was observed, every territory was visited several (3–5) times during July–October. The maximum number of unringed birds observed that escaped capture was not more than 23 individuals during the whole period of study, but the number of birds that escaped observation altogether is probably higher. There were gaps in the monitoring efforts in some years (1978, 1980 and 1983), and a few pairs escaped attention because of insufficient coverage of the study area in other years, especially 1984–87 (details given by Lillandt 1993). When artificial feeding started in new study areas, it took more than a season to locate all the jays, because some birds needed a long time to find and learn to visit feeding stations. Altogether, information was obtained from 456 group-years.

Genetic analyses

Microsatellite loci

We used a set of nine polymorphic microsatellite loci (Table 1), one of which was cloned from the Siberian jay genomic library. Eight loci were found by amplification of loci isolated in other species; in three of these the primer sequences needed modification before successful amplification in the Siberian jay. The new or modified primer sequences as well as details about amplification conditions will be published elsewhere (see also Hansson et al. 2000).

DNA extraction

DNA from 158 blood samples was extracted according to a standard protocol including proteinase K, phenol-chloroform and ethanol precipitation (Sambrook et al. 1989). In feathers collected from 261 individuals, approximately 5 mm of the base of the shaft was cut into strips with a paper knife. This was put into 400 μ l lysis buffer (0.1 M Tris-HCL pH 8.5, 0.005 M EDTA, 0.2 % SDS, 0.2 M NaCl; Laird et al. 1991) with 12 μ l proteinase K (10 mg/ml) for digestion at 56 °C overnight, followed by standard phenol-chloroform treatment and ethanol precipitation. The edge part of the paper knife was renewed between feather samples to avoid DNA contamination across samples. DNA extracted from blood was stored in 1xTE buffer. DNA concentration was estimated by spectrophotometry and samples were

Table 1. Microsatellite loci used for parentage determination in the Siberian jay. New primer sequences and other details on amplification conditions will be published elsewhere.

Locus	source	No. of alleles	H_O	H_E	Parentage exclusion probability	
					First parent	Second parent
Ck.1B5D	Tarr & Fleischer 1998	2	0.552	0.497	0.123	0.187
Ck.2A5A	Tarr & Fleischer 1998	16	0.754	0.751	0.375	0.554
CkL5	mod. fr. Tarr & Fleischer 1998	11	0.854	0.820	0.476	0.649
LTML7	mod. fr. McDonald & Potts, unpubl.	2	0.411	0.403	0.081	0.161
LTML8	mod. fr. McDonald & Potts 1994	14	0.864	0.844	0.525	0.691
MJG1	Li et al. 1997	2	0.461	0.431	0.093	0.169
Per1	Siberian jay, Lillandt et al., unpubl.	6	0.547	0.537	0.159	0.322
Ppi1	Martinez et al. 1999	4	0.595	0.542	0.150	0.298
Ppi2	Martinez et al. 1999	5	0.768	0.737	0.317	0.492

H_O = observed heterozygosity, H_E = expected heterozygosity.

diluted to 25 ng/ μ l for PCR reactions. DNA from feathers was stored in 25 μ l H₂O (without quantification), because of some amplification problems apparently caused by the TE-buffer (Jackson et al. 1991).

PCR reactions of 10 μ l included 25 ng genomic DNA from blood samples or 1–3 μ l from the total amount of 25 μ l DNA dilution from one tail feather.

Data analysis

Total allele frequencies, observed heterozygosities and expected heterozygosities for the pooled data set from all 419 individuals were calculated with the software ‘Cervus’ (Marshall et al. 1998). With the same computer program we also calculated the probability of excluding a randomly chosen individual from parentage both in cases where no parent is known (‘first parent’ test) and cases where one parent is known (‘second parent’ test). Despite a reasonable theoretical exclusion power of the marker system (based on the assumption of panmictic populations the exclusion probabilities were 0.945 and 0.993 for ‘first’ and ‘second’ parent, respectively), the large genetic similarities between close relatives in the population prevented us from a successful search for mothers and fathers separately from the whole population of established birds. A test with the software ‘Cervus’, presuming that neither parent is known (as is normally the case with juvenile jays sampled in summer–autumn), gave several or even many potential parental candidates. The available microsatellite markers were insufficiently polymorphic to allow parentage to be assigned with a satisfactory level of significance with this method. The parameters used for simulation and the outcome of the analysis are summarised in Tables 2 and 3.

Table 2. Parameters used in the simulation for maternity inference in Siberian jays with the software ‘Cervus’.

Parameter	Value used
Number of candidate females	35
Proportion of candidate females sampled	0.9
Proportion of loci typed	0.97
Rate of typing error	0.005
Number of tests	10,000
Relaxed confidence level	80 %
Strict confidence level	95 %

Table 3. The success of maternity inference using ‘Cervus’ for 298 Siberian jay juveniles sampled in the study populations in western Finland 1976–98. The analysis was performed as a ‘first parent’ test, with only females included as parental candidates. The observed number of obtained maternity with 80 % and 95 % confidence are shown along with the predicted numbers from the simulation, and the critical value of D.

Number of maternities	80 %	95 %
Observed	160 (54%)	44 (15%)
Predicted	181 (61%)	68 (23%)
Critical value of D	0.78	2.23

As an alternative strategy, we first tested genetic monogamy in cases where the female attending the nest was found to be the mother of the nestlings (20 birds, from 15 clutches, ringed as nestlings and DNA-sampled as juveniles, for which both parents were sampled). The problem that potential extra-pair fertilisations could pass undetected, especially if the extra-pair male is a close relative of the male attending the nest (Double et al. 1997), was checked by testing 15 real cases. For each nestling we combined the nest attending mother with every sampled male living in the same population during the year the nestling was ringed (2–16 potential extra-pair males/nestling, totalling 90 combinations), and counted the number of ‘novel bands’ in the nestling, i.e. the number of alleles that could not have been inherited from that particular male or from the nest attending mother. The proportion of total matches with ‘wrong’ males describes the power of the paternity exclusion system for this ‘second parent’ test. As a comparison we also combined the nest attending mothers with males sampled in the neighbouring populations during the same year and counted the number of ‘novel bands’ in nestlings in this situation (243 combinations). Only birds for which we had complete microsatellite data (nine loci) were included in these tests.

To check further the possibility that the nest attending female was not the mother of the nestlings because of egg-dumping, we performed another test for females, in which no information concerning potential fathers was included. In this test we compared every one of the same 15 nestlings with all other females within the same population (104 comparisons) and between po-

pulations (263 comparisons), and counted the frequency of mismatches in the nestlings (i.e. the number of loci in which the nestlings had no allele that could have been inherited from the tested female). The proportion of total matches with 'wrong' females describes the power of the testing system for females as 'first parent' in these kin-structured populations.

After confirming monogamy we examined each one of the 298 sampled juvenile birds by performing a search for their potential parents among all the established pairs that were found or could have been found in the same population during their hatching year. On territories where one or both parental birds had been replaced between monitoring in subsequent autumns, every possible combination of parents was tested as a potential parental pair. Pairs not observed in a particular year, but that could have been undetected in the territory where they were found later, were also included as potential parents in the comparisons. If no parents were found in the same population, juveniles were also checked against established pairs in the neighbouring populations during the same year. In families for which we had incomplete allelic information, parentage determination was performed only if the level of variation on scored loci allowed this to be done, i.e. if one parental pair could be unambiguously found.

As it has been shown that theoretical exclusion probabilities based on assumptions of panmictic populations are severely overestimated if the studied population is in fact kin-structured (Double et al. 1997), we also investigated the reliability of our parentage determinations by examining overall allelic mismatch frequencies within and between populations. By comparing every juvenile bird (with complete microsatellite data, $n = 261$) with every potential, completely scored parental pair in the same population during the same year (1–36 pairs, altogether 4,040 comparisons), we obtained an estimate of the proportion of total matches 'by chance' (i.e. the proportion of juveniles with an allelic composition matching more than one parental pair) and information about the distribution of mismatch frequencies. A smaller set of 489 comparisons was also made between 84 juveniles and parental pairs from different populations, to investigate the distribution of mismatch frequencies in a situation where the problem of relatedness should be smaller.

To test the assumption of total genetic monogamy further we analysed the distribution of mismatch fre-

quencies among those juveniles that did not match any parental pair (30 individuals, out of which 24 were completely scored) and checked how many mismatching alleles these birds had at a minimum. If these young were the results of extra-pair fertilisations the mismatch frequency should be similar to the situation where the fathers of nestlings with known mothers were 'replaced'; if the mismatches were caused by mutations or typing errors the minimum number of mismatches should be low. But if their parents were not sampled, or if the juveniles were immigrants from other populations, the mismatch frequency should be similar to the outcome of the comparisons between juveniles and non-parental pairs within or between populations. We also checked the 49 juveniles found not to be offspring of their 'social parents' (i.e. the established pair with which they were associated) analysed as a pair, for the possibility that one parent could have been a genetic parent while the other one had been cuckolded or replaced. Note that this group of 49 birds is not exactly the same as the later mentioned group of 50 juveniles that had left their natal territory before sampling, because some of the 50 birds were not found together with an established pair.

Results

Microsatellite typing and inheritance patterns

In total we successfully scored 96.8 % of all loci (including sex determination, unpublished data) in the 419 individuals, despite the fact that we had collected only one tail-feather from 130 of them. In the 261 individuals from which we had only feather samples we successfully amplified at least three microsatellite loci in all cases, and in 87.7 % of them at least eight loci were successfully scored. Samples from feathers more than 10 years old resulted in fewer amplified loci (Fig. 1.) and weaker amplified fragments than samples from feathers collected in the 1990s, which mostly gave amplification products indistinguishable from results based on blood samples.

Mendelian inheritance was confirmed in 10 families (15 different clutches), where 20 sampled juveniles had been ringed as nestlings. The presence of a null allele was obvious in the Ck.2A5A locus; a female that seemed homozygous for a rare allele did not transfer this allele to two juveniles (not ringed as nestlings), that

mean number of mismatching alleles in the offspring when fathers were replaced within a population was 2.3 and if the candidate fathers were taken from a neighbouring population it was 3.2. In females the ‘first parent’ test showed that in 12 cases out of 104 within-population comparisons another female totally matched the nestlings when no information about the fathers was included (exclusion probability 0.885, Fig. 3). In comparisons between populations the corresponding value was 15 out of 263 (0.943, Fig. 3, close to the theoretically calculated value 0.945). The mean number of mismatching alleles in female-nestling comparisons within populations was 1.9 and between populations 2.1.

Finding parental pairs

The results of parentage determinations are summarised in Table 4. Altogether, we found totally matching parental pairs for 268 of the 298 sampled juveniles (89.9 %). Among these 268 juveniles there were 30 whose parents had been incompletely sampled. In 21 cases we lacked DNA samples from one of the social parents. However, these juveniles perfectly matched the other social parent and no other sampled pair in the population during the same year. In nine cases we lacked DNA samples from both social parents, but four of these were ringed as nestlings. None of the nine juveniles was found to match any other sampled parental

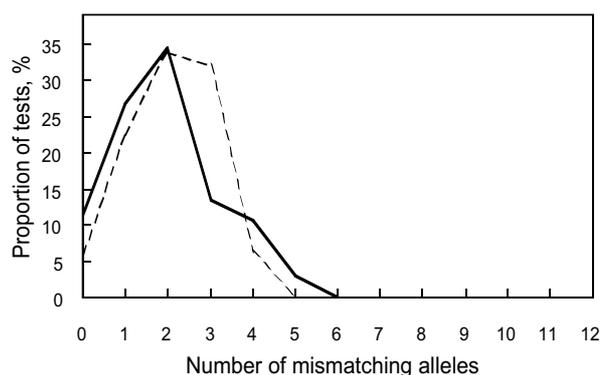


Figure 3. Numbers of mismatching alleles in Siberian jay nestlings, when tested against females other than the nest attending female, assuming that the father is unknown (‘first parent’ test). The lines indicate the proportion of cases with different numbers of mismatching alleles when candidate females were taken from the same population (104 comparisons, solid line) or from neighbouring populations (263 comparisons, broken line). Zero mismatching alleles indicate that another female could have been the mother of the nestling without being detected by our parentage testing system. Only completely scored (9 loci) individuals were included.

pair, hence it is likely that their social parents were also their genetic parents. Twelve juveniles (of which seven were completely scored) that totally matched their so-

Table 4. Results of parentage determinations in 298 Siberian jay juveniles, when their allelic profile was compared to observed parental pairs in the populations studied in western Finland 1974–98. Parentage assignment presumed that no allelic mismatches between the juvenile and the parental pair occurred. Juveniles were divided into two groups: (a) juveniles that totally matched their social parents in the autumn (if they were sampled); and (b) juveniles that did not match their social parents or that had no potential parents in the same territory.

	Matched no sampled pair	Matched only one pair	Matched two pairs	Total
(a) Juveniles observed with matching parents				
Both social parents sampled		176	7 + 5 ⁽¹⁾	188
Only one social parent sampled	21 ⁽²⁾			21
None of the social parents sampled	9 ⁽³⁾			9
(b) Juveniles observed after leaving natal territory				
	30	47	2 + 1 ⁽⁴⁾	80

⁽¹⁾ In seven cases the allelic data were complete, the rest were based upon 3–8 compared loci.

⁽²⁾ All 21 birds matched the only sampled social parent; four of them were ringed as nestlings.

⁽³⁾ Four of these nine were ringed as nestlings.

⁽⁴⁾ In two cases the allelic data were complete; one was typed on only four loci.

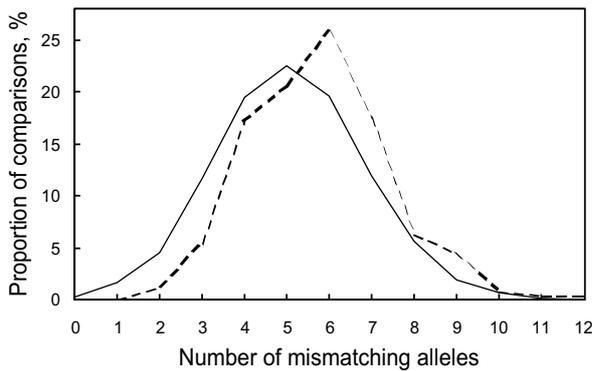


Figure 4. Numbers of mismatching alleles in completely scored Siberian jay juveniles sampled in the autumn, when comparing them with every potential parental pair except their presumed parents. The lines indicate the proportion of cases with different numbers of mismatching alleles when juveniles were compared to pairs within the same population (261 juveniles, 4,040 comparisons, solid line) or from the neighbouring populations (84 juveniles, 489 comparisons, broken line). Zero mismatching alleles (in within-population comparisons, $n = 9$) indicate that the juvenile perfectly matched an additional pair besides the presumed parents.

cial parents did also match another pair. Among the 268 juveniles for which matching parental pairs were found, there were 50 individuals (18.7 %) that had left their natal territory before sampling. In 47 of these one totally matching parental pair living in another territory could be identified, while in only three cases (two of which were completely scored) two matching parental pairs were found. In these three cases it is not possible to determine which one of the two matching parental pairs included the true genetic parents. For the remaining 30 juveniles (10.1 %), no matching parental pair could be found in the whole study area.

In 4,040 comparisons between 261 completely scored juveniles and potential parental pairs within the same population, we found 9 cases where the allelic composition of all nine loci perfectly matched two different parental pairs; i.e. a perfect match with one pair that could not have been the genetic parents (Fig. 4). All of these cases were found in the northern study area during the years 1994–98, and, according to pedigree data, in five cases one or both pair-mates were closely related with the other perfectly matching pair-mates. The average number of mismatching alleles between a ju-

venile and all breeding pairs present during its hatching year was 5.1, while the proportion of cases with only one mismatched allele was 1.7 % and with two mismatches 4.6 %. When comparing 84 juveniles to parental pairs in the neighbouring population (489 comparisons) the average number of mismatching alleles was higher, 5.8, and no total matches with ‘wrong’ parents or any one-allele mismatches occurred, while the proportion of cases with two mismatches was 1.2 % (Fig. 4).

The analysis of the minimum number of mismatching alleles in all the 30 juveniles for which no parents were found (Fig. 5) showed that four birds had a mismatch in only one scored locus compared to at least one parental pair. Two of these four birds were scored on eight, one on five and one on all nine loci. Five more birds had at least two mismatching alleles, while 21 juveniles had a minimum number of 3–6 mismatching alleles in every comparison. The average number of mismatching alleles in the 24 completely scored juveniles that did not match any parental pair, when compared to potential parental pairs in the population where they were ringed, was 5.6 (Fig. 6), which is close to the value 5.8 obtained in the between-population ‘simulation’ above.

A more detailed analysis of the 49 juveniles observed with an adult pair that was not their genetic parents supported the view that they were non-offspring that had joined the group rather than young from extra-pair

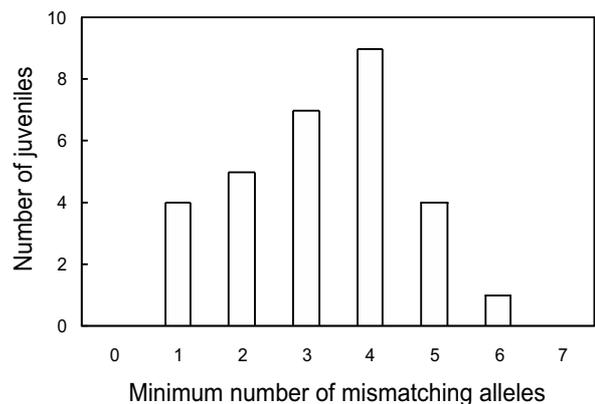


Figure 5. The minimum number of mismatching alleles found when comparing all the 30 juvenile Siberian jays for which no parents were found with every potential parental pair within the same population, regardless of the number of scored loci (4–9 loci, mean 8.1).

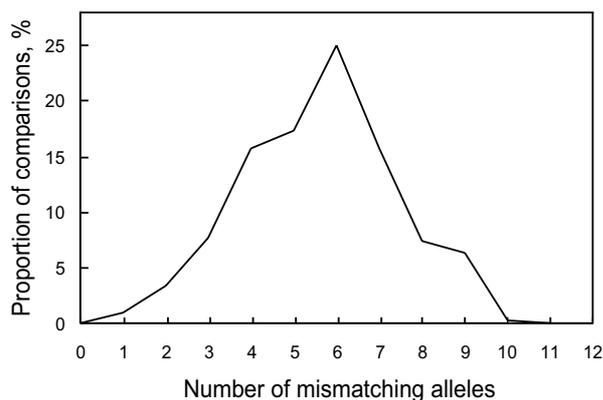


Figure 6. Numbers of mismatching alleles in completely scored Siberian jay juveniles, that did not match any parental pair ($n = 24$), when comparing them with every potential parental pair within the same population. The line indicates the proportion of cases with different numbers of mismatching alleles.

matings. Thirty-seven of these (75.5 %) did not match either of the two adults, while the remaining 12 could have been an offspring of one of the social parents. In seven cases the juvenile matched the male, but not the female, and in only four cases the female but not the male. In one case the juvenile matched both adults when compared to them separately, but did not match when they were combined.

Discussion

Microsatellite analysis

This study demonstrates the power of microsatellites as markers for parentage testing in complicated data sets. Because microsatellite analysis is PCR-based it was possible to make parentage determinations based on feather samples collected over a long period of time. However, we experienced increasing amplification problems in feathers more than 10 years old, suggesting a slow degradation of the DNA in feather samples when stored at room temperature. The microsatellites allowed us not only to check parentage within observed groups, but also to find the parents of juveniles that had moved away from their natal territory before sampling. This would not have been possible had we used mini-

satellite DNA fingerprinting, nor is it possible to run DNA fingerprinting using feather samples.

Parentage determination

Nine polymorphic loci with a total theoretical exclusion probability of 0.945 for 'first parent' and 0.993 for 'second parent' were not enough to enable a search for mothers and fathers separately, the reason being insufficient allelic variation between close relatives living contemporaneously in the population. A similar situation has been found in an Australian marsupial, the northern hairy-nosed wombat *Lasiorhinus krefftii* (Taylor et al. 1997), where insufficient variation at nine polymorphic loci, combined with incomplete sampling and missing demographic information, prevented parentage determination. Double et al. (1997) examined the same kind of problem in the superb fairy-wren *Malurus cyaneus*, an Australian cooperatively breeding bird with several male relatives living in clusters. Their study showed that the 'paternity exclusion probability' calculated assuming panmictic populations will be severely overestimated if the population is kin-structured.

To circumvent these problems we applied another strategy, based on the assumption of genetic monogamy in the population. As we had quite complete information about the established pairs each year, we were able to search for parents among pairs instead of evaluating potential mothers and fathers separately. By doing so we greatly increased the power of the parentage testing system (Meagher & Thompson 1986), and were able to find matching parental pairs to 268 of the 298 sampled juvenile birds. For three juveniles (among these 268) that had left their natal territory before sampling, two matching pairs were found, and therefore the genetical parents could not be determined. We failed to find any matching parents for only 30 sampled juveniles (10.1 %) during the whole 25-year study period.

Possible error sources in parentage determinations

Extra-pair fertilisations

We were able to confirm genetic monogamy in 15 clutches (20 nestlings), a sample size that is too limited to detect rare cases of extra-pair fertilisations. However, similar results have been presented by Ekman et al. (1994), based on DNA-fingerprinting. Despite the high

theoretical exclusion probability for ‘second parent’ (0.993) in our study, the estimated paternity exclusion probability was not more than 0.90 (see Double et al. 1997). According to our ‘simulation’ with replaced fathers in observed families, we found that extra-pair fertilisations would in most cases produce offspring with a low number (1–2) of mismatching alleles. There were only nine such juveniles among those for which we failed to identify the parents, and in only four of them there was a mismatch at only one locus. Furthermore, of the 49 birds found not to be offspring of their social parents in the autumn, only five matched the social mother, and for four of these we found another matching parental pair. Altogether, these results strongly suggest that extra-pair fertilisations could at most account for only a few cases of failed parentage determination among all the 298 juveniles evaluated in this study.

Female egg-dumping

All of the tested nestlings perfectly matched the female attending the nest, giving no indications of female egg-dumping in this species. The exclusion power of our analyses for females as ‘first parent’ was 0.885, according to our within-population ‘simulation’, and 0.943 for females from other populations. The latter value is very close to the theoretically calculated exclusion probability (0.945) for ‘first parent’, assuming panmictic populations. Because female Siberian jays exhibit longer dispersal distances than males (pers. obs.), neighbouring females are not close relatives as frequently as neighbouring males. The risk that egg-dumping performed by neighbouring females would pass undetected is thus smaller than in the case of extra-pair fertilisations. Both on basis of the genetic analyses and our own field observations we find the occurrence of female egg-dumping to be very improbable in this population.

Null alleles, mutations and typing errors

In one family we observed a null allele at one locus. However, calculations of observed and expected heterozygosities suggest that null alleles are rare at all loci, despite the fact that most of the primers were developed for other species. Because our parentage determinations extended over as many as eight successive generations, many individuals were included first as offspring and later as parents. We therefore had repeated opportunities to detect occasional mismatches cau-

sed by null alleles, point mutations or typing errors in particular individuals. Nevertheless, only one apparent null allele was found. Because most juveniles were observed together with their potential parents, we were able to check for mismatches on occasional loci during the typing process, and thereby minimise the occurrence of typing errors. Our within-population ‘simulation’ showed that in only 1.6 % of the comparisons a juvenile mismatched a non-parental pair in only one allele, i.e. occasional mutations or typing errors are very unlikely to cause false parentage determinations.

Incomplete information about pair-bonds during the breeding season

In 32 group-years (7.0 % of the observed 456 group-years) we lacked DNA samples from both parents. There were also an undetermined, low number of unchecked groups as a result of gaps in the monitoring efforts during some autumns, and possibly there were cases where territory holders had been replaced twice between subsequent autumns, and thereby some breeding pairs escaped attention. These three sources of missing information certainly contributed to the number of individuals for which no parents were found (30 juveniles), but they should not affect parentage determinations in the other juveniles.

Two statistical problems

In the analyses we were faced with two different statistical problems: (1) checking parentage within observed family groups, and (2) searching for the parents of juveniles that were not ringed on their natal territory.

(1) Our within-population ‘simulation’ showed that the probability that a randomly chosen juvenile from the same population would perfectly match a non-parental pair is very low; this happened in only one case out of 449 comparisons (0.22 %). Many, or possibly all, of the matched non-parents were close relatives to the correct parents, suggesting that the risk of multiple matches is connected to the occurrence of large kin-clusters in the population (unpublished data). The probability of multiple matches is therefore higher for individuals hatched within successful ‘clans’, while the risk of immigrants being mistakenly regarded as offspring is very low.

(2) Among the 50 juveniles for which matching parents were found in a territory other than that in which

the juvenile was ringed, it is hard to evaluate the reliability of the parentage determinations. However, we found only three cases (two of which were completely scored) of total matches with two different pairs in these comparisons. The risk of mistakenly assigned parentage when presuming total matching between parents and offspring is therefore still small, even if it is considerably larger when systematically searching through the whole population than when testing only within observed groups.

Considering the potential error sources discussed above we expect that the risk of not finding the parents, due to mismatching alleles caused by mutations, null alleles, typing errors or extra-pair paternity, is larger than the risk of falsely assigning parentage. The fact that we found very few such slightly mismatching individuals, however, implies a low impact of these errors.

To conclude, we find the examined microsatellite typing system to be sufficiently reliable for making inferences about parentage in the Siberian jay populations studied here. Every line of evidence supports the assumption of genetic monogamy in this species, and the high success of parentage determinations that we achieved would not have been possible had extra-pair fertilisations been frequent. However, this study shows that reliable parentage determination in kin-structured populations demands a large number of polymorphic microsatellite loci, especially if there is no a priori information about who is the mother, or about parental pair-bonds.

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