SHORT COMMUNICATIONS

Sequential Polyandry in the Common Redpoll (*Carduelis flammea*)

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Polyandry is usually defined as the mating of a female with more than one male during the course of a breeding season (Oring 1982, 1986; see Ford 1983 for a more restrictive definition). Oring (1986) recognized two main types of polyandrous mating systems, classical and cooperative polyandry. Both are rare among avian species, and particularly among passerines (Jenni 1974, Oring 1982) where most reported cases are based on limited and sometimes doubtful data (Ford 1983). Recent field studies of color-marked populations have nevertheless provided good evidence for the regular occurrence of cooperative polyandry in the Dunnock (*Prunella modularis*; Burke et al. 1989), classical polyandry in the American Goldfinch (*Carduelis tristis*; Middleton 1988), and simultaneous polyandry-polygyny in Smith's Longspur (*Calcarius pictus*; J. V. Briskie pers. comm.). Isolated cases of sequential polyandry have been reported also in the Northern Mockingbird (*Mimus polyglottos*; Fulk et al. 1987), the Black-capped Chickadee (*Parus atricapillus*; Waterman et al. 1989), and *Geospiza* finches (Boag and Grant 1984).

We documented a case of polyandry in the Common Redpoll (*Carduelis f. flammea*), a species whose mating system has never been carefully studied. Using Jeffreys' DNA fingerprinting probe 33.15 (Jeffreys et al. 1985a), we have confirmed that the two adults attending the first nest were the biological parents of the nestlings, and at the second nest we observed that the first male participated in neither mate guarding during nest construction nor mate provisioning during brooding. Apart from Middleton's (1988) American Goldfinch study (and possibly Stokes' [1950] American Goldfinch population, see Middleton 1988: 299), there is no other report of polyandry in cardueline species, even in well-studied taxa (e.g. Newton 1973). We speculate that polyandry may be more prevalent in redpolls than our single observation indicates.

Seutin observed redpolls in Churchill, Manitoba, 27 May to 1 August in 1988, and 1 June to 6 July in 1989. Nests were found usually by searching spruces (*Picea balsamea*) and stands of willows (*Salix* spp.). We mist-netted adults attending nests, banded them with standard USFWS metal bands and colored celluloid bands (A. C. Hughes, Middlesex, England), took a blood sample, and made standard morphometric measurements.

To extract DNA from the blood samples of the female involved in the polygamous relationship, her

first mate, and the two nestlings from her first nest, we used an Applied Biosystems nucleic acid extractor as described by Seutin et al. (1991). Aliquots (4 µg) of redissolved DNA were cleaved with 15 units of the restriction enzymes *Alu*I, *Hae*III, and *Mbo*I for 3 h at 37°C in the appropriate buffers. Restriction fragments were separated on a 30 cm long 0.8% agarose TBE (0.09 M Tris, 0.09 M boric acid, 0.002 M Na$_2$-EDTA, pH 8.0) gel running at 1.3 V/cm for 50 h. Restriction fragments resulting from the digestion of lambda DNA with *Hind*III were used as molecular size markers. Electrophoresed DNA fragments were depurinated, denatured, and neutralized *in situ* and then transferred to a charge-modified blotting membrane (Gene Screen Plus®; DuPont) as described by Seutin et al. (1991). The blot was dried, baked at 80°C for 2 h, and incubated overnight at 65°C in a sealed plastic bag with 15 ml of prehybridization solution (Westneat et al. 1988).

An aliquot (25 ng) of Jeffreys' 33.15 probe (Jeffreys et al. 1985a) was labeled with alpha ^{32}P-*d*CTP in a random priming reaction to a specific activity of 1.1 × 10^9 dpm·µg^{-1}, added to the prehybridization bag, and hybridization proceeded at 65°C for 12 h with shaking. The blot was then washed two times for 10 min in a 2× SSC, 0.1% SDS solution at room temperature, and two times for 30 min in a 2× SSC, 0.5% SDS solution at 65°C with constant shaking. Restriction fragments having homology to the probe were visualized by autoradiography at −70°C for 6 days on a Cronex® film with one Cronex® intensifying screen.

Behavioral observations.—On 18 July 1988, two Common Redpoll nests were found ca. 200 m apart. The first nest (nest A) contained two large nestlings 8–9 days old. The second nest (nest B) consisted of only a few dry twigs of spruce and willow intermingled with pieces of dry grass; its construction had probably been started on the previous evening. No adult was seen close to either nest.

The next day (19 July) at 0930, two adult birds were seen arriving close to nest B. The male (i.e. the bird that was not carrying lining material) had no red coloration on the breast and sides, and it was therefore assumed to be a second year (SY) bird (Molau 1985, Seutin et al. 1989). The second bird was carrying ptarmigan feathers and was assumed to be female. She went to the nest, sat for approximately 10 min, and when she flew off, she was followed immediately by the male that was perched in a willow 1 m away. This behavior is typical of male cardueline involved

in mate guarding (e.g. Newton 1973; G. Seutin pers. observ.). No other redpoll was seen nearby.

Later the same morning, a male and a female were captured and color-banded at nest A. The female was captured as she left the nest when Seutin approached. Based on the shape of their central rectrices, we determined both birds to be more than two years old (ASY; Molau 1985); the male was a typical red-breasted bird.

On the next visit, 21 July at 0600, nest A was empty. The adult male and the fledglings were seen neither that day nor during the following week (6 h spent within 500 m of the nest). At 0700, nest B was found unoccupied, completely lined and containing 1 egg. After 8 min of observation at nest B, the female that was color-marked at nest A arrived at the nesting bush carrying lining material; an unbanded SY bird simultaneously arrived some 15 m away. The birds did not move significantly for 9 min, at which point the watch had to be terminated.

On 27 July, at 1100, nest B contained a full clutch of 5 eggs. During a 1-h watch, the banded female did not leave the nest and was provisioned once by an unbanded SY bird. A 45-min watch on 31 July yielded one feeding of the brooding female by an unbanded SY bird. At the end of both watches, the female was flushed so that her identity as the female previously attending nest A was confirmed. Several other redpolls were observed on both days, but not the male that was color-banded at nest A.

Seutin left Churchill on 1 August before the eggs in nest B hatched so we have no data on the fate of that nest. It was found empty in 1989. Some nests, known to have been abandoned in 1988, were found in 1989 still containing eggs, and nests from previous years were found in 1988 and 1989 containing whole eggs, eggshell fragments (a sign of clutch predation), or dead nestlings of all ages. Neither nest was used in 1989, and none of the 1988 adults or nestlings were seen in 1989.

Assessment of parentage through DNA fingerprinting. — On the DNA autoradiograph (Fig. 1), it is possible to match every band present in an offspring with a band present in one of the adults. Two bands were considered to match when they had the same electrophoretic position and similar autoradiographic intensity. Assuming the probability of band sharing between unrelated individuals in the population to be 0.20 (see below), the probability that two unrelated individuals will share 8, 9, or 11 bands as did the adults and offspring (Table 1) is 2.4×10^{-6}, 4.7×10^{-7}, and 1.8×10^{-8} (Jeffreys et al. 1985b). These results indicate that both adults are related to the offspring and are probably their parents. The actual relatedness of individuals can be estimated from the similarity between their DNA fingerprints, using the statistic $D = 2N_{AB}/(N_A + N_B)$, where N_A and N_B are the number of fragments scored in individuals A and B, and N_{AB} is the number of fragments shared by both (Jeffreys

et al. 1985b, Wetton et al. 1987). The overall similarity between the adult patterns is 0.20 (Table 1). This value is similar to that for the comparison of unrelated individuals with the same or similar probes in humans and several avian species (Burke and Bruford 1987, Morton et al. 1990, Meng et al. 1990, P. T. Boag and B. N. White, unpubl. data). It is considered as the probability of band sharing between unrelated redpolls. $D_{\text{cumulative}}$ between adults and offspring ranged from 0.53 to 0.64 (Table 1). These values are very close to that expected for a parent-offspring pair, $D = 0.58$ (Jeffreys et al. 1985b, Burke and Bruford 1987). Because we have no knowledge of the segregation pattern of the individual autoradiographic bands, some assumptions involved in the calculation of the expected D-value might be violated (Lynch 1988). We feel, however, that if violations exist, they involve only a small proportion of the resolved bands and that our estimate is realistic. We conclude that the two adults caught near nest A are the biological parents of the nestlings. A genetic assessment of parentage of the female's second brood could not be done because the male attending nest B could not be caught for blood sampling, and the clutch was not hatched when Seutin left Churchill.

In their reviews of polyandry in birds, Jenni (1974) and Oring (1982) mentioned seven passerine species for which polyandry has been reported. Ford (1983) judiciously pointed out that most of these reports are based on equivocal data or are cases of mate switching between breeding attempts (see Ford [1983] for a discussion of sequential monogamy with mate switching vs. sequential polyandry). Unequivocal evidence for the regular or occasional occurrence of polyandry in wild passerines has now been collected for a few species (see above).

Our evidence for the occurrence of sequential polyandry in the Common Redpoll is as follows. An ASY female was observed on the same day feeding almost fully grown nestlings at one nest (nest A) and building a second nest (nest B) 200 m away. DNA fingerprints showed that the female and a color-banded ASY male were the biological parents of the offspring in nest A. That male was never observed close to nest B; instead, an unbanded SY bird accompanied the female both times she was observed bringing nesting material to nest B, and a similar bird (presumably the same putative mate) was later observed twice feeding the brooding female. Unfortunately, we did not have the DNA samples to evaluate the relative role of the two males in the female's second clutch. Whether or not the second male fathered the second clutch (in part or in whole), our observations indicate polyandry at least from a behavioral point of view.

There is no previous report of polygamy in the Common Redpoll or in the closely related Hoary Redpoll (*C. hornemanni*). This is not surprising because only two color-marked populations of these species have been studied, and in both cases with limited
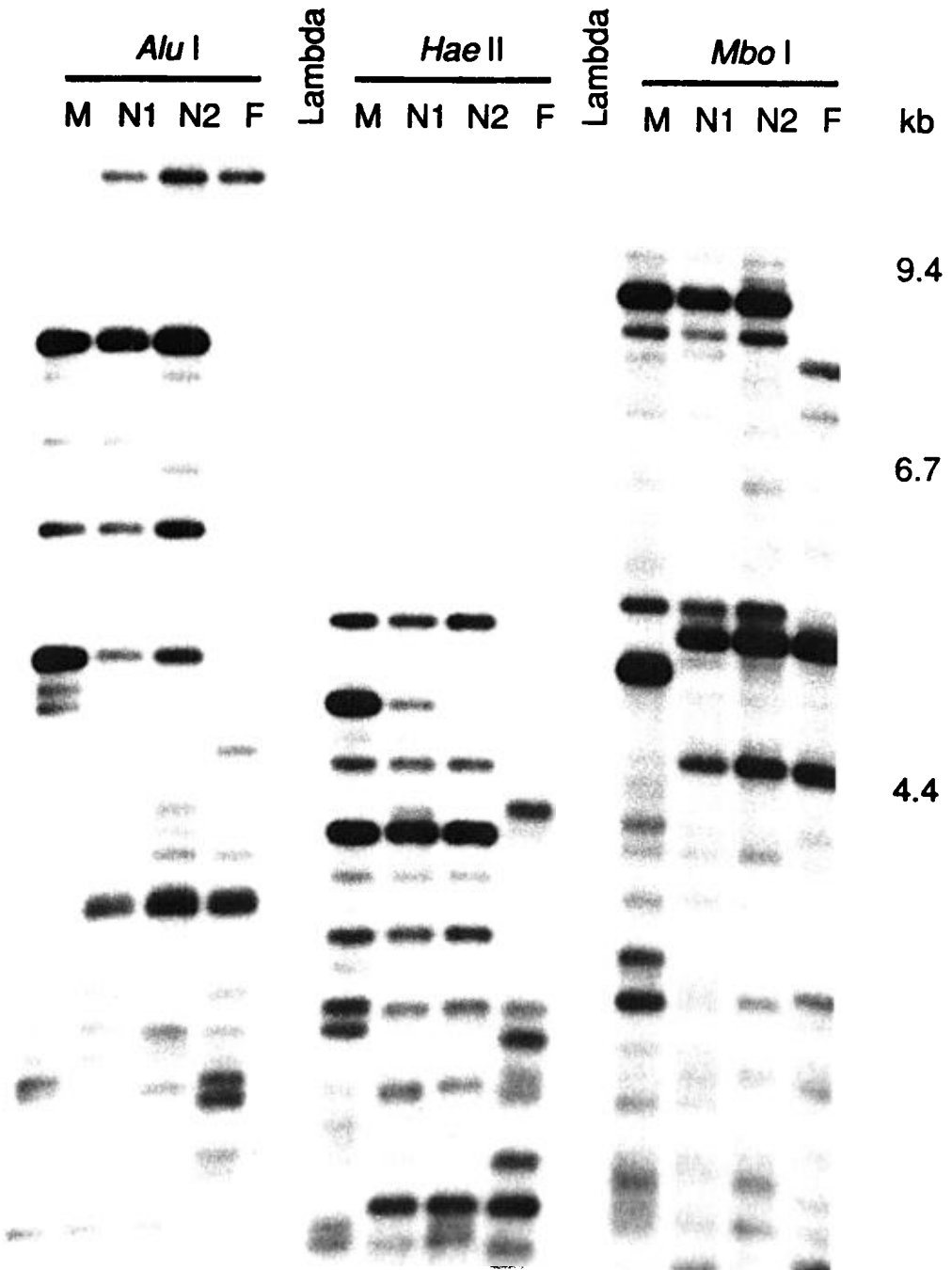
Fig. 1.   DNA fingerprinting of a Common Redpoll family using Jeffreys' 33.15 probe. DNA samples (4 μg) were digested with *AluI, HaeIII,* and *MboI.* All bands present in an offspring (N1 and N2) are matched by a band of similar mobility and intensity in one or both parents (M: male; F: female). Some faint bands that could be scored on the autoradiographic film are not visible on this print. Poor resolution precluded scoring the lower bands in the *MboI* lanes. Lambda DNA digested with *HindIII* was used as molecular size marker; units of calibration are kilobases (kb).

effort and for a limited time (Nyström and Nyström 1987; G. Seutin unpubl. data). Further, study of successive breeding attempts of individual redpolls is difficult because nests are usually not reused for second clutches (Jehl and Smith 1970, G. Seutin unpubl. data), and successive nests are probably built some distance apart. Circumstantial evidence indicates that individual female redpolls may even raise broods hundreds of kilometers apart during a single breeding season (e.g. Peiponen 1957).

Middleton (1988) reported sequential polyandry in a population of the American Goldfinch as a regular alternative mating tactic present at low frequency in a predominantly monogamous population. His report is most interesting in the context of our observation in the Common Redpoll as the two species are congeners, share several attributes of their breeding biology, and in both cases polyandry was noticed in females with previous breeding experience. Middleton (1988) suggested that polyandry in goldfinches evolved from monogamy through increasing male parental care and female desertion (Oring 1986). His model requires a male-biased sex ratio in the population.

As presented, Middleton's (1988) system strongly benefits females and second males. By switching mates, females increase their probability of successfully rearing a second brood if time is limiting, and second males gain the opportunity to leave progeny in a season where they were initially unpaired. First males have the advantage of mating with experienced females, who in the goldfinch have a higher reproductive success than younger females (Middleton 1979), but first males also risk being abandoned with the offspring. Middleton (1988) suggests that this risk is offset by the greater chance of survival of the male's genetic investment obtained through breeding with an experienced female.

In redpolls, females have the capacity to double-clutch (Peiponen 1957, Troy and Shields 1979, this report). Because they face a short breeding season, there is a great advantage for them to start their second nest as early as possible, possibly through sequential polyandry as presented by Middleton (1988). We have no strong evidence that a male-biased sex ratio exists in redpolls, that ASY females have a higher fertility than SY females, or that experienced males are able to singlehandedly rear young to independence; all these conditions are necessary in Middleton's model. Nevertheless, we feel these conditions are probably met—at least in some years in some populations—and consequently, Middleton's (1988) model will apply to redpolls as well as to goldfinches.

It is hard to determine the frequency at which polyandry might occur in redpolls because, in both our population and in the literature, there are no other data on the identity of the successive mates of an individual female during a breeding season. Indirect evidence suggests that polyandry might be more fre-

TABLE 1. Number of bands scored (N) and percentage of shared bands (D) in the DNA fingerprinting analysis.[a]

	AluI	HaeIII	MboI	Cumulative
N_M	18	19	20	57
N_F	18	11	15	44
N_{O1}	19	17	19	55
N_{O2}	18	15	19	52
D_{MF}	0.17	0.20	0.23	0.20
D_{MO1}	0.49	0.72	0.72	0.64
D_{MO2}	0.61	0.71	0.62	0.64
D_{FO1}	0.59	0.50	0.47	0.53
D_{FO2}	0.50	0.46	0.65	0.54

[a] M: male; F: female; O1: offspring 1; O2: offspring 2.

quent than our single record indicates. We observed single males caring for fledglings more frequently than we observed single females, a fact that might reflect females' desertion and involvement in second breeding attempts with other males. Of the family groups with adults that were observed or mist-netted in Churchill in 1988 and 1989, four were accompanied only by a male (all ASY males, 2 Common and 2 Hoary redpolls), one group by a female (a SY Common Redpoll), and two groups by both parents. This distribution is not statistically different from a uniform distribution (expected ratio 1:1:1; $G = 1.83$, $P > 0.1$), and it might have resulted from causes other than female desertion, but it is in the direction expected if polyandry occurs at a significant frequency. Further, the single males that attended fledglings were all ASY birds as expected in Middleton's (1988) model.

We feel that polyandry might be more prevalent in cardueline finches (including redpolls) than is generally recognized, especially in species with short or unpredictable breeding seasons. That it has been reported in only two cardueline populations probably reflects the lack of within-season site fidelity of females in several species (e.g. Newton 1973).

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The Enigma of Multiple Nest Building by Male Marsh Wrens

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Male Marsh Wrens (*Cistothorus palustris*) build multiple domed "dummy" nests on their territories, which collectively comprise the "courting centers." Males sing and display to females from these nests (Welter 1935). Females usually build the "breeding" nests in which eggs are laid (Welter 1935). Male Marsh Wrens continue to build nests even after females have begun to incubate. Marsh Wrens frequently mate polygynously, although the proportion of polygynously mated males in a population varies from marsh to