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STEPFATHER DETECTED BY DNA FINGERPRINTING IN BUSHTITS (Psaltriparus minimus)

USE OF SIMILARITY COEFFICIENTS FROM DNA FINGERPRINTING TO ASSESS RELATEDNESS IN BUSHTITS (Psaltriparus minimus)

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Stepfather detected by DNA fingerprinting in bushtits

Use of similarity coefficients from DNA fingerprinting to assess relatedness in bushtits

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Abstract

The bushtit (Psaltriparus minimus) of Southeastern Arizona is a cooperative breeder, in which 35% of nests have more than two birds attending. Helpers join nests at all stages of the breeding season, and potentially make genetic contributions to the nest. Behavioural observations suggest that the fluid social system of the bushtit may provide opportunities for extra-pair fertilizations (EPFs) and intra-specific brood parasitism (ISBP). DNA fingerprinting was applied to assess parentage in the 1988 and 1992 breeding seasons. Using three minisatellite probes (Jeffreys 33.15, 33.6 and PER), fingerprints were generated from nine complete families (nestlings and adults) from 1992 and twelve families of nestlings, in this study, from 1988. Parentage analysis of the 1992 families indicated strict genetic monogamy, despite the fact that two of the breeding groups were socially polyandrous. A case of serial monogamy in a double-brooded nest provided evidence of a male helper achieving reproductive success after aiding in the rearing of non-kin in the first brood. This result suggests the potential reproductive benefits of helping behaviour in a double-brooding species. Genetic relatedness, within groups of nestlings from the same brood, was analyzed in the 1988 samples. In using the 1992 data as a calibration, the 1988 broods were found to contain no unrelated

dyads resulting from ISBP. Discrimination between full and half-sibling relationships was less clear. The analysis of relatedness serves as a useful comparison to other studies that have used a multiple probe approach to assess relatedness.

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Preface

The bushtit (Psaltriparus minimus) is one of the three species described in the original paper entitled "Helpers at the nest" by Alexander Skutch (1935). Helping behaviour in bushtits may vary with geographical location. In contrast to a California study, work conducted on bushtits in southeastern Arizona found multibird nests to be quite common. Sloane's work in the Chiricahua Mountains suggested that approximately 35% of the nests in this area had extra birds. Some of these extra birds were clearly 'helpers', owing to the fact that they did not contribute genetically to the nest, and therefore fed nestlings that were not their own. Helpers joined nests at all stages of the breeding season, and in some cases there may have been genetic contributions to these nests (before egg laying). The fluid social structure of the bushtit breeding flock provides opportunities for extra-pair fertilizations (EPFs) and to a lesser degree, intra-specific brood parasitism (ISBP). Greater interest has been given to the importance of EPFs and ISBPs with the realization that they can be integral evolutionary elements in the breeding system.

This research follows the five year study conducted by Sarah Sloane on the bushtit population in the Chircahua Mountains of southeastern Arizona. The previous study documented the

incidence of multibird nests and developed evolutionary and ecological determinants of this breeding system. I have applied DNA fingerprinting to assess parentage and relatedness in this population. The genetic profiles of putative parents (based on behavioural observations) were compared with those of the nestlings, to assign or exclude parentage. Nine complete bushtit families from the 1992 breeding season were analyzed to assess parentage in this year. This season's data will be discussed in terms of the importance of double-brooding and it's implications on reproductive strategies.

The second chapter concerns the determination of genetic relatedness in twelve families of nestlings from the 1988 breeding season. The methodology was based on the Piper and Rabenold (1992) methods which assessed the suitability of band-sharing analysis for assigning relatedness to pairs of individuals in the stripe-backed wren (Campylorynchus nuchalis). In theory, these methods enable one to assign pairs of unknown relatedness (eg. first-order relatives, second-order relatives or unrelated pairs) to classes of relatedness based on similarity between observed and expected distributions of band-sharing scores.

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Literature Review

The aspects of a social organization that determine the ways in which males and females come together for breeding, the number of mates, the characteristics of pair bonds and the patterns of parental care by each sex are collectively referred to as the mating system (Emlen and Oring, 1977). Trivers (1972) observed that mating systems are not cooperative ventures where males and females raise offspring in perfect harmony. Each individual should behave to maximize it's own success even if this is at the expense of it's mate.

Monogamous breeding systems are characterized by each breeding adult mated to one member of the opposite sex. Lack (1968) suggested that approximately 90% of all bird species are monogamous. He believed that monogamous mating systems were predominant because biparental care was the best strategy for maximizing lifetime reproductive success. Contrary to this, removal experiments in some bird species, suggest that monogamy may be due to the limited opportunities for polygyny. Strong competition among males, and female vigilance against polygyny are constraining factors on polygynous opportunities (Davies, 1991).

In birds, the role of parental care and territory quality are significant in the evolutionary transition between monogamy and polygyny. In evolving to a polygynous system, a female must accept a reduced role of the male in feeding her offspring. If this loss is

small relative to the gain achieved by mating with a male with a high quality territory, then polygyny will be advantageous for her (Brown, 1975). Alternatively, polygyny may arise through males monopolizing females by controlling territory resources. In this case, polygyny can cost females by decreasing their access to resources (Davies, 1991).

In some cases, the term monogamy may well describe the social system but not necessarily reflect their genetic contribution to future generations (Mock, 1983; Gowaty, 1985). In many monogamous bird species, males adopt a 'mixed reproductive strategy', not only guarding their own female and helping her to raise a brood, but also attempting to copulate with other females (Trivers, 1972). Females may accept extra-pair copulation simply because it is costly to resist them, or alternatively, females may solicit matings from a high quality male, or to gain the help of a second male in the care of her offspring, which would then be defined as both social and genetic polyandry (Davies, 1991). Females can also increase their reproductive success by dumping eggs into the nests of conspecifics, termed intra-specific brood parasitism (ISBP).

Cooperative breeding is a type of social system in which one or more members of a social group provide care to young which are non-descendants. The care given usually includes providing food but may also involve other parental-type behaviours as well. Although the existence of helpers has been known in avian systems for many

years (Skutch, 1935), cooperative breeding has been studied in detail only over the last two decades (Stacey and Koenig, 1990).

Emlen (1990) summarized the conceptual framework of cooperative breeding systems by posing the following two questions: Why do helpers not breed on their own? and; Why do such individuals help? When constraining factors make breeding very costly, offspring may delay dispersal and breeding by remaining with the natal group. Territory limitation or habitat saturation are often the constraining factors, such that occupancy of a territory is a prerequisite for independent breeding, then auxiliaries must wait until an established breeder dies or is displaced. Stacey and Ligon (1987) have proposed an alternative hypothesis stressing the benefit of philopatry which increases the auxiliary's chance of gaining a territory by inheritance or enabling earlier detection of an available territory.

In many species of cooperative breeders there is a significant adult sex ratio bias. An excess of males has been reported in many bird species, which increases the competition for female mates. These demographic constraints on independent breeding may increase the incidence of helpers (Emlen, 1991).

The "altruistic" behaviour exhibited by helpers is most often founded on a social bond, such as occurs between parent and offspring, monogamous pair or expanded family group (Brown, 1975). Hamilton's (1964) theory of inclusive fitness states that genotypes causing individuals to aid others who are likely to carry the same

genes may increase in frequency in a population. Indirect fitness benefits derived from helping non-descendent kin can occur in two ways, by increasing the survival of breeders and/or the current breeding effort. To confirm these predictions, it must be established that the activities of helpers significantly increases the fitness of breeders and that the helpers are closely related to the breeders (Emlen, 1991). Work by Emlen and Wrege (1988) on white-fronted bee-eaters (Merops bullockoides) provides the strongest evidence for indirect fitness gain from helping. Bee-eater helpers have a major effect on the reproductive success and show nepotistic favouritism in their decisions of whom to aid.

Direct fitness benefits to helpers can also be derived from; 1) enhanced probability of survival; 2) increased reproductive success when it does become a breeder, and 3) enhanced likelihood of becoming a breeder in the future (Emlen, 1991). Survival enhancement can come from the increased size of the social group, resulting from greater reproductive success, and it has been shown that individual survival is higher in larger groups (Gaston, 1978). Also, the probability of morality is lessened by remaining in the familiar area rather than risking early dispersal (Emlen, 1991).

Increased reproductive success resulting from helping is based on experience gained by being a helper which can improve parental care later. If breeding requires the development of specialized skills prior experience may be a prerequisite for successful reproduction (Brown, 1987). Woolfenden and Fitzpatrick (1984)

provide indirect evidence that male Florida scrub jays (Aphelocoma c. coerulescens) increased their reproductive performance by helping in previous years.

Helping behaviour can increase direct fitness benefits by increasing the likelihood of becoming a future breeder. There is evidence to support the notion that helping can be critical to the future acquisition of the natal area (Woolfenden and Fitzpatrick, 1990). In many species of cooperative breeders, there is an imbalance in the adult sex ratio, leading to constraints on members of the limited sex in becoming breeders. Reyer (1980) proposed that helping could establish social bonds that enhanced the likelihood of helper pairing with the opposite sex breeder in the future. In the pied kingfishers (Ceryle rudis), secondary helpers greatly increase their chance of pairing in the following year in this manner. Fitness calculations on secondary helpers demonstrate how beneficial it can be to help as opposed to sitting out for a year (Reyer, 1980).

The bushtit (Psaltriparus minimus) is tiny passerine found in the family Paridae. Bushtits occupy a variety of habitats in Western North America ranging from British Columbia to Central America and as far east as Texas (Peterson, 1990). During the summer and fall, bushtits associate in small compact flocks usually consisting of 15 to 30 individuals, but occasionally as high as 200 (Baldwin, 1933). Early in the breeding season, which extends from late-February to late-July, bushtits build nests of grass, moss, spider webs and egg

casings, feathers and other materials. Four to seven eggs are laid in mid-April. Young hatch after a fourteen day incubation period and remain in the nest for an additional fourteen days before fledging (Sloane, 1992).

Bushtits are one of the three species described in Alexander Skutch's (1935) paper entitled "Helpers at the nest". Helping behaviour in bushtits may vary with geographical location. Bushtits observed in Guatemala appeared to be nesting in polygynous and polyandrous groups (Skutch, 1935). A later study by Ervin (1974) in California revealed a very low incidence of multibird nests (1/147). This one case of helping behaviour was concluded to be anomalous (Ervin, 1977): a case of mistaken identity (Jamison and Craig, 1987) or redirected behaviour (Emlen, 1981). In contrast to the California study, a study conducted on bushtits in southeastern Arizona found multibird nests to be quite common. Sloane's work in the Chiricahua mountains suggested that approximately 35% of the nest in this area had extra birds. Some of these extra birds were clearly 'helpers', owing to the fact that they could not have contributed genetically to the nest, and therefore fed nestlings that were not their own. These extra birds, or supernumeraries, were most often males derived from the pool of unmated males (owing to a male biased sex ratio) failed breeding attempts.

Supernumeraries joined nests at any stage of the breeding season, and in some cases there may have been genetic contributions to these nests. The fluid social structure of the bushtit breeding

flock provides opportunities for extra-pair fertilizations (EPFs), and to a lesser degree, intra-specific brood parasitism (ISBP) (Sloane, 1992). The bushtit social system is quite similar to that of the long-tailed tit (Aegithalos caudatus). Failed breeders of this species join at existing nests with potential genetic contributions (Gaston, 1973; Glenn, 1985).

Given that helping behaviour is so prevalent in the Arizona population of bushtits, one might expect some selective advantage to be associated with helping. Sloane suggests that the key to the observed behaviour may have a physiological basis. Bushtits apparently do not have physiological adaptations for extreme cold, in spite of the fact that they encounter low temperatures throughout much of their range (Chaplin, 1982). Huddling behaviour has been shown to increase heat retention in bushtits such that distances between roosting birds decrease in response to a decrease in temperature (Smith, 1972). Even at 20°C, paired and huddling bushtits each consume only 79% of the energy expended by a lone bushtit (Chaplin, 1982). Lone birds or birds which have lost their nest to predation early in the breeding season, when the weather is still cold and unpredictable, need to find a nest quickly to avoid dying of exposure (Sloane, 1992). Under such thermal conditions, the incidence of multibird nests would be expected to increase as supernumeraries "pay rent" by aiding in rearing of offspring in return for a place in the nest during the night.

A similar physiological limitation in green woodhoopoes (Phoeniculus purpureus) has been proposed as an important factor in the evolution of their cooperative breeding system. The poor thermoregulatory abilities of woodhoopoes restricts them to roosting in holes, which are limited and unpredictable in distribution (Ligon et al., 1988). As a result, unmated individuals will often join breeding groups for access to a roosting hole.

Bushtits that breed in the Chiricahua Mountains commonly have two broods in a single breeding season. Double-brooding provides opportunities for reproductive strategies associated with the helping to raise the first brood to increase the likelihood of being a breeder of second brood. Reyers (1980) observations of pied kingfishers where many secondary helpers aid in one year and assume primary breeding status in the next, provide evidence that such reproductive strategies are present in cooperative systems. In the bushtit system, unpaired males may help in the first brood in order to increase their likelihood of becoming the primary breeder in the second brood.

With the potential for many reproductive tactics present in a system, the importance of realized reproductive success becomes more evident. Greater interest has been given to the roles of EPFs and ISBPs with the realization that they can be integral evolutionary elements in the breeding system. The possible occurrence of successful EPFs and/or ISBPs may severely bias estimates of lifetime reproductive success which is of great importance for the

evaluation of mating strategies and social organization (Gullberg et al, 1992).

With the advent of molecular genetic technology, behavioural ecologists now have a means of directly assessing the reproductive success of individuals in a population. Birds are ideally suited to DNA analyses because their nucleated erythrocytes provide an accessible and abundant supply of nuclear DNA with a small blood sample (Quinn and White, 1987). The variable DNA sequences that are most commonly used in studies of mating behaviour are random restriction fragment length polymorphisms (RFLPs) and minisatellite DNAs (DNA fingerprinting). The RFLP method involves the detection of genetic polymorphisms at one locus at a time (Quinn et al, 1987), whereas multilocus DNA fingerprinting simultaneously detects polymorphisms at multiple loci (Burke, 1989).

The genetic profiles generated by means of DNA probes that detect hypervariable regions in the genome, provide individual specific patterns of DNA fragments (Jeffreys et al, 1985). DNA fingerprinting has been applied to breeding systems on two fronts: parentage analysis (Burke and Bruford, 1987; Burke et al, 1989; Westneat, 1990; Gibbs et al, 1991); and estimating genetic relatedness (Piper and Rabenold, 1992; Burke and Bruford, 1987; Rabenold et al, 1990, Quinn et al., in press).

This study follows the five year investigation conducted by S. A. Sloane on the bushtit population in the Chiricahua Mountains of

southeastern Arizona. Sloane's study documented the incidence of multibird nests and developed evolutionary and ecological determinants of this breeding system. I have applied DNA fingerprinting to assess parentage and relatedness in this population.

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Stepfather detected by DNA fingerprinting in Bushtits (*Psaltriparus minimus*).

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Abstract

Parentage was assessed in a population of Bushtits (Psaltriparus minimus) using DNA fingerprinting. The Bushtit breeding system is highly variable, including paired and multibird nests as well as single and double-brooding. Parentage, based on three multilocus minisatellite probes, was assigned for 9 breeding groups composed of 6 paired, 2 polyandrous and one serially monogamous double-brooded group. All of the 10 broods examined, consisting of 59 nestlings, contained offspring of a genetically monogamous pair. The probability of misassigned parentage due to an undetected extra-pair fertilization (EPF) or intra-specific brood parasitism was 8.1×10^{-11} and 3.0×10^{-27} respectively. The male which fathered the second brood in the serial monogamous nest was observed feeding unrelated nestlings of the first brood. The genetic data, along with the behavioural observations, provides evidence of 'stepfatherhood', which can be viewed as a reproductive tactic in this species. The importance of reproductive options provided by double-brooding are discussed in light of the genetic data.

Introduction

Monogamous breeding systems were thought to occur in over 90% of bird species (Lack 1968). One variant is cooperative breeding, which is characterized by aid-givers contributing parental care to offspring that are not their own. These aid-giving individuals may be non-breeding adults (helpers) or co-breeders which share reproduction with same sex group members (Stacey and Koenig 1991). Hamilton's (1964) theory of kin selection has been used by some to explain the evolution of systems where aid-giving individuals are not contributing genetically to the breeding effort. In contrast to related helpers, non-kin helpers do not receive the inclusive fitness benefits derived from investing in relatives. Under such circumstances, the importance of ecological constraints or other non-adaptive factors has been proposed as an explanation (Stacey and Koenig, 1990).

Bushtits (Psaltriparus minimus) are one of the first species described as cooperative from observations of helpers at the nest (Skutch 1935). Bushtits breeding in the Chiricahua Mountains of Arizona display notable variation with respect to breeding group composition. Previous studies have determined that approximately one third of the nests have more than two attending adults (Sloane 1992). A male biased sex ratio exists in this population ranging from 1:1.2-1:1.5. The helpers are predominantly unmated males or birds that have failed in breeding attempts (Sloane 1992). In approximately 19% of nests, helpers have been observed to join prior

to or during the egg laying stage, thereby providing the opportunity for genetic contributions. In addition, the relatively high incidence of double-brooding in these birds (Sloane pers. comm.) may give additional reproductive options to helpers. Genetic studies are necessary to determine if these birds are achieving reproductive success.

DNA fingerprinting has become a very useful tool for behavioural ecologists. The development of DNA probes (Jeffreys et al. 1985) which detect high levels of genetic variation by hybridizing to tandem repeats in the nuclear genome has enabled genetic discrimination of closely related individuals. Many studies have demonstrated the power of DNA analysis when applied to parentage determinations in avian breeding systems (Quinn et al. 1987; Wetton et al. 1987; Burke et al. 1989; Birkhead et al. 1990; Gibbs et al. 1990; Westneat 1990; Gullberg et al. 1992; Dunn et al. 1993; Quinn et al. in press). Genetic evidence indicates that reproductive strategies of non-exclusive mating are common in many bird species. Studies have indicated that extra-pair fertilizations (EPFs) and intra-specific brood parasitism (ISBP) are important sources of reproductive success (Quinn et al. 1987; Wetton et al. 1987; Burke et al. 1989; Birkhead et al. 1990; Gibbs et al. 1990; Westneat 1990).

Here we use DNA fingerprinting to investigate parentage in a population of Bushtits in the Chiricahua Mountains. By describing the characteristics of the breeding groups and determining the

patterns of parentage we attempted to elucidate reproductive strategies at work in the Bushtit breeding system.

Materials and Methods

Field Methods

The study was conducted during the 1992 breeding season in the Cave Creek basin of the Chiricahua Mountains within the Coronado National Forest (31° 51'N, 109° 15'W). This population of Bushtits was studied by S.A.S. between 1986 and 1991. The site is at an elevation of approximately 1700-1800m and characterized by open oak woodland with Arizona white oak (Quercus arizonica), emory oak (Q. emoryi), alligator-bark juniper (Juniperus deppeana), and border pinyon pine (Pinus discolor).

The 4 km^2 study site was monitored from March through July. Nest searches were performed twice weekly by transecting the site and listening for Bushtit calls. Once a bird was heard it was sighted, and followed to the nest. Consistent encounters with the study birds during searches suggested that all nesting in the study site was detected. All adults observed feeding nestlings were captured in mist nets. In cases where one male and one female were captured at a nest they were regarded as the putative parents. Adults and nestlings were banded with individual specific

combinations of 3 plastic colour bands and a U.S.F.W.S. numbered aluminum band.

Blood samples of 50μl were collected by brachial venipuncture and stored in lysis buffer (4 M Urea; 0.2 M NaCl; 0.1 M Tris-HCl, pH 8.0; 0.5% n-lauroylsarcosine; 0.01 M CDTA). The brachial vein was lanced with a 27 guage needle and the blood was collected in a heparinized capillary tube. Samples were obtained from 9 complete families, consisting of 20 adults and 59 nestlings (two broods were sampled from the same nest). Nestlings were sampled at 12-13 days of age (approximately 2 days before fledging) and immediately returned to the nest.

Molecular Methods

Genomic DNA was extracted from blood stored in lysis buffer. Between 10 and 25 μl of blood was mixed in 3.0 ml of lysis buffer. Protein was digested at 37°C using two additions of 100 μl (80 units) of proteinase K and gently rocked for 24 hrs. Samples were extracted twice with phenol/chloroform (70:30), once with choloroform and then ethanol precipitated. The DNA was dissolved in 0.2 to 0.6 ml of TNE_2 (0.01 M Tris-HCl, 0.01 M NaCl, 0.002 M EDTA, pH 8.0) and quantified by flourometry and agarose gel electrophoresis.

Approximately 10 μg of DNA was digested for 4-5 h with the restriction enzyme Hae III. The cut DNA was precipitated in 70%

ethanol and 0.3 M sodium acetate and redissolved in 20 μl of TNE_2. Four μg of sample DNA was combined with 3 ng of a DNA cocktail of lambda digested with Hind III/EcoR I and BstE II as a control for possible differential mobility between samples (Galbraith et al. 1991), and loaded in a 0.8% agarose gel. Electrophoresis was performed at 1.2-1.5 V/cm for approximately 45 h. The DNA was transferred with 10X SSC (3 M NaCl, 0.3 M sodium citrate, pH 7.0) by Southern blotting to charged polyvinylidene difluoride membrane (Immobilon-N) which was air dried and then baked at 80°C for 1-2 h.

Blots were incubated for 2-12 hours at 65°C in prehybridization solution (7% SDS, 0.001 M EDTA, 0.26 M sodium phosphate, pH 8.0 and 1% bovine serum albumin; fraction V; Westneat et al. 1988). Minisatellite sequences 33.6 and 33.15 (Jeffreys et al. 1985) and the mouse probe pSP2.5RI (PER; homologous to the Drosophila periodic locus; Georges et al. 1988) were used sequentially as hybridization probes. Probes were labelled by random primer extension (Pharmacia oligolabelling Kit; >8X10^8 cpm/ug) with ^{32}P-dCTP and hybridizations run overnight at 65°C. Blots were then washed in 2X SSC, 0.1% SDS; for 15 mins. at room temperature, for 15 mins. at 65°C, and for 30 mins. at 65°C. X-ray film, with intensifying screens, was exposed to the radioactive blot at -70°C for 1 to 14 days. We stripped blots with 0.4 M NaOH at 42°C for 30 min for repeated probings. Following probing with fingerprint probes, the membranes were probed with lambda, to provide size markers.

The banding patterns of offspring were compared, or scored, with those of the putative parents. Bands were considered to be the same if their relative intensities were similar (if less than twice the intensity) and if the position of the bands were the same using internal size markers as guides (Galbraith et al. 1991).

After scoring, the statistic $\underline{D}=2N_{AB}/(N_A+N_B)$ was calculated where N_{AB} is the number of bands shared by both birds and N_A and N_B are the number of bands found in lane A or B, respectively (Wetton et al. 1987). The $\underline{D}$-value, which represents the proportion of bands shared between individuals of a dyad, varies from zero, when no bands are shared, to one, when all bands are shared. The means of $\underline{D}$-scores will be given ± S.D as outlined in Lynch (1990).

Results

Families

Six of the 9 families studied were attended by a pair of adults. The other three breeding groups (P2, #204, and #207) had variable conformations. Group P2 was the only breeding unit which had more than two adults directly involved in chick rearing. Two males and one female were observed feeding nestlings at this nest. Nest #204 consisted of a single pair at the early stages of breeding (through to incubation). The male at this nest disappeared and was never seen during the chick feeding stage. The female, that was observed feeding the chicks, was attended by two replacing males.

Neither of these males was observed feeding at the nest. The last nest (#207), produced two broods in the course of the breeding season. The first brood was attended by a pair during egg laying and incubation, but again the resident male disappeared and was not observed beyond this stage. Chicks of the first brood were fed by the resident female and a replacing male. The replacing male remained with the female through to the fledging of the second brood.

Fingerprints

The three probes used revealed highly variable banding patterns. The DNA fragment size ranges hybridizing to PER, 33.15 and 33.6 were 2.2 - 13, 2.2 - 20 and 2.2 - 16 kb respectively. A proportion of bands were detected by more than one probe. The extent of duplicated detection of fragments between probes were as follows: 0.22 and 0.16 for PER vs 33.15; 0.21 and 0.18 for PER vs 33.6; and 0.19 and 0.23 for 33.15 vs 33.6. The number of scored fragments detected by the probes PER, 33.15 and 33.6 were $\bar{X}$ = 18.7 ±4.9; $\bar{X}$ = 16.1 ±3.9; and $\bar{X}$ = 13.0 ±4.8 respectively. Some fragments detected in individual offspring were not found in either parent (N=7). These fragments, presumed to arising from mutations, occurred at a rate of 0.002 per fragment. Average band-sharing ($\underline{D}$) among unrelated adults was 0.172 for PER, 0.155 for 33.15 and 0.219 for 33.6.

In all of the ten broods analyzed, nestlings within a brood were parented by a genetically monogamous pair. The band-sharing scores of full-sibling and parent-offspring dyads are in the range expected for first-order relatives (Table 1). No kin relationships between adults of a breeding group were found to exist. Based on this, the probability that parentage was misassigned due to an undetected EPF was 8.1×10^{-11} or to an undetected ISBP event was 3.9×10^{-27}. This was calculated as the probability that background band-sharing explains a nestlings' paternally inherited bands arising from an undetected EPF, or all of a nestlings bands due to an undetected ISBP.

Despite the presence of more than one male in nests P2 and #204, each brood was fathered by a single, but different male. Figure 1 illustrates the fragment profiles of the socially polyandrous trio and seven nestlings from nest P2 for the three probes. The mean band-sharing scores, across the probes, between the assigned father (male 2) and the 7 nestlings were 0.543, 0.511, 0.513, 0.614, 0.565, 0.517 and 0.502 compared with those between male 1 (helper) and the nestlings, which were 0.258, 0.245, 0.151, 0.256, 0.260, 0.293 and 0.180. Based on number of exclusionary bands (paternally inherited bands not present in a male), male 1 was eliminated as a potential father of these nestlings, although this individual was observed providing parental care by feeding at the nest.

The fingerprints of the #207 group verify that the female at this nest was serially monogamous (Figure 2). Mean band-sharing scores between the female and the two broods were 0.687 ±0.073 for the first brood and 0.691 ±0.042 for the second. The father of the first brood disappeared following egg laying. The mean band-sharing score between this male and the seven nestlings of the first brood was 0.603 ±0.066. This brood of seven nestlings was fed by the resident female and a 'replacing' male. This second male, or stepfather, was excluded as the father of the first brood as the mean number of exclusionary bands, across the three probes, per nestling was 19.1 ± 4.2. The stepfather male which was mist-netted at the nest while feeding the first brood, fathered the second brood of three nestlings. The three nestlings of this brood had a mean band-sharing score of 0.701 ±0.045 with the second male. Nestlings of the first brood (N1 - N7) are maternal half-siblings to the three nestlings (N8 - N10) of the second. The twenty-one dyads (7X3) of half-siblings in Table 1 were derived from this family.

Discussion

Genetic monogamy has been observed in other bird species. DNA fingerprinting has revealed no extra-pair offspring in the following species: Fulmars (Fulmarus glacialis; Hunter et al. 1992); Florida Scrub Jays (Aphelocoma coerulescens; Quinn et al. in prep); Willow Warblers (Phylloscopus sibilatrix; Gyllensten et al. 1990).

The social system of Bushtits is considerably different from that of other birds where parentage is monogamous. Sloane's (1992) work indicates that these birds have a system that could be best described as helpers-at-the-nest where helpers are aiding non-kin. Between 1986 and 1990, one-third of the nests observed had multibird breeding groups attending them and both colour banding (Sloane 1992) and DNA evidence from this study indicates that helpers are unrelated to the rest of the breeding group.

Serial monogamy in the double-brooded nest suggests that a reproductive strategy may underlie the 'helping' behaviour. The 'stepfather', which invested in the non-kin nestlings in the first brood, might have been described as an altruistic individual had there not been a second brood. The observed helping behaviour can be viewed in a new light given that fitness benefits were received. Reproductive gains, relative to the possible alternative option of not breeding, were realized by this individual, inviting speculation into the role of breeder replacement in the evolution of the breeding system. Similar reproductive tactics have been observed in secondary (unrelated) helpers in Pied Kingfishers which frequently assume primary breeding status by replacement, and in doing so increase the fitness benefits associated with delaying breeding effort (Reyer 1991).

The importance of replacement mates, or birds that adopt unrelated offspring, has been expounded by Rohwer (1986). In his review of this phenomena, Rohwer outlines circumstances which

would favor adoption over infanticide in order to maximize future reproductive success by replacement mates. Adoption can only be favoured within a breeding season when the potential for double-brooding exists. To date, the issue of double-brooding has received little attention with regard to cooperative breeding systems.

In typical breeding seasons in the Chiricahuas, Bushtits will attempt a second brood if the first was fledged without any significant delays (Sloane, pers comm). Double-brooding would be prevented only if there was a late onset of breeding or if the first attempt was predated. Therefore, it follows that the second brood provides a major source of reproductive success to Bushtit breeders. Under these circumstances, helpers may be "waiting in the wings" for an opportunity to assume the primary breeding role in the second brood. Double-brooding can add another important dimension to the evolution of aid-giving behaviour when inclusive fitness benefits are not involved.

This study raises questions concerning the importance of double-brooding in cooperative breeding species. Our data, which are derived from a year of relatively low nesting density, suggest that significant reproductive opportunities may be realized by helpers when a second brood is an option. In years when nesting density is higher, the incidence of multibird nests and double-brooding would be more prevalent. Under such conditions, reproductive options to helpers would increase.

Acknowledgments

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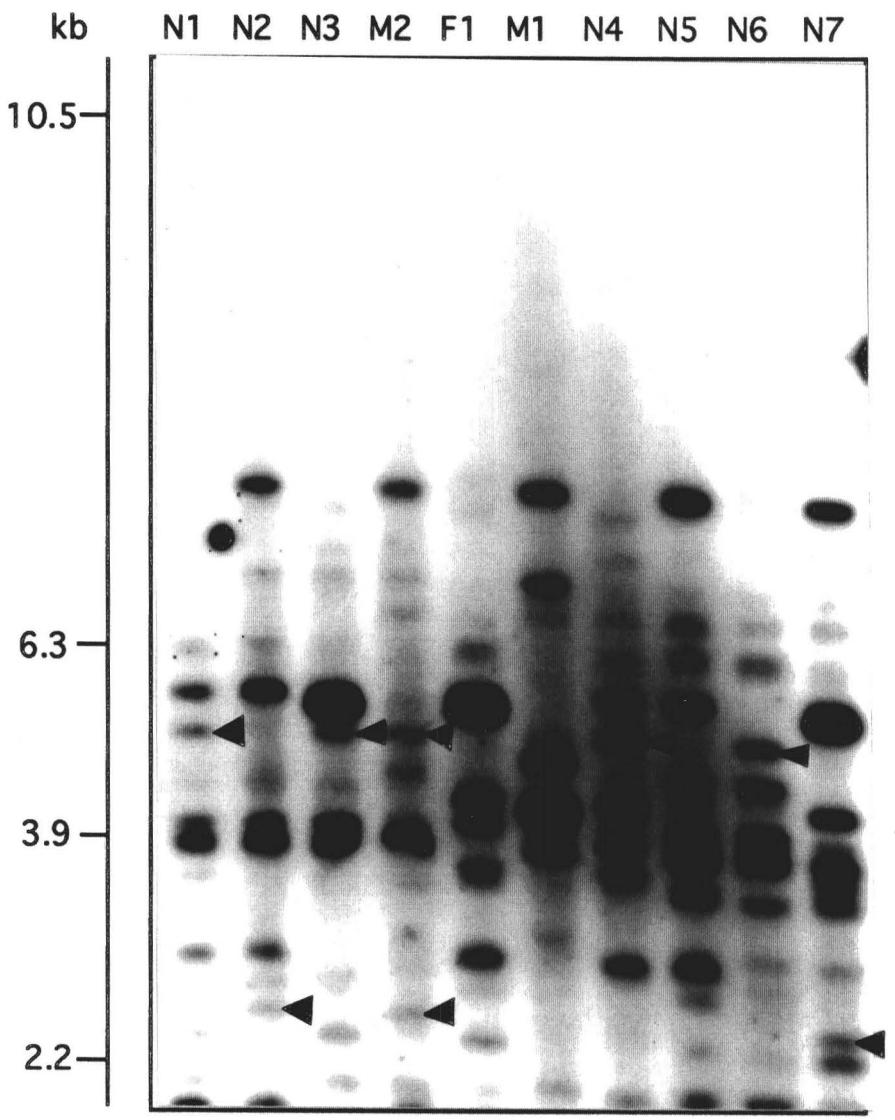
Table 1: D-scores of DNA fingerprints of dyads with different relationships, across three probes and the mean score across the probes.

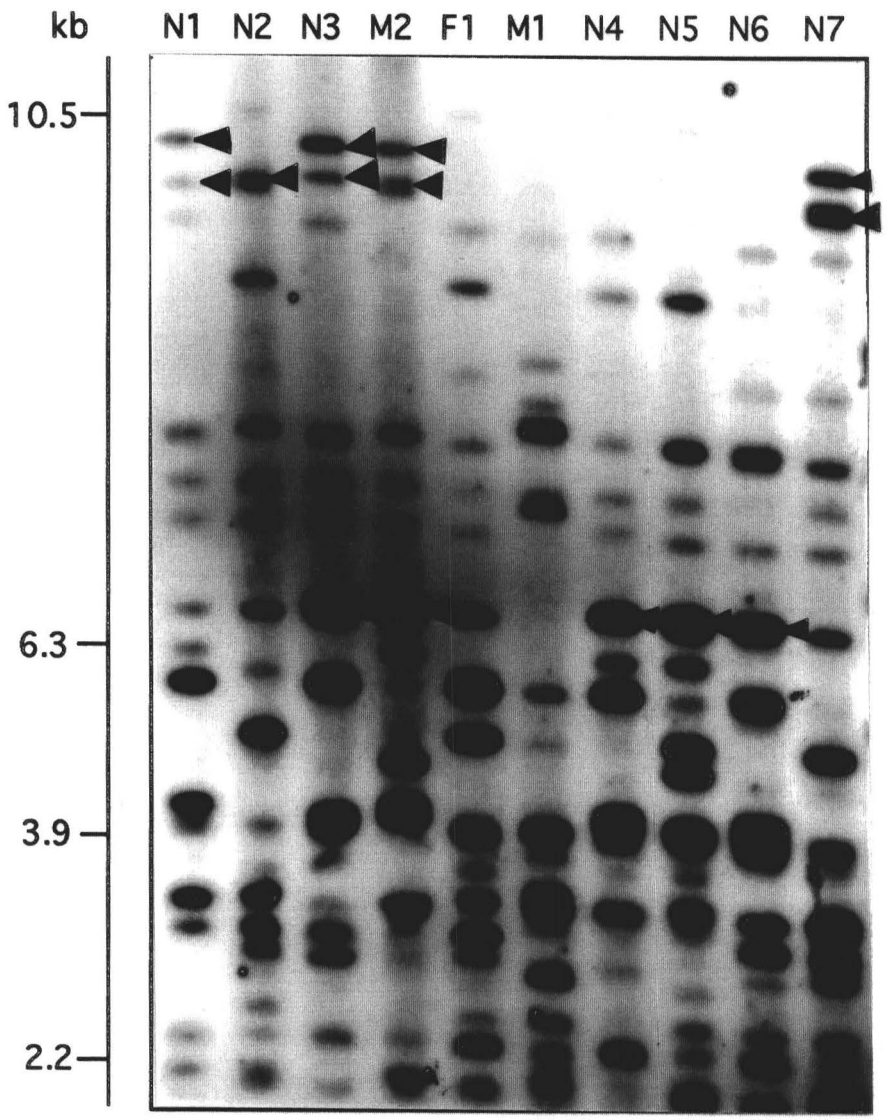
Relationship	N(dyads)	PER $\bar{X} \pm$ S.D.	33.15 $\bar{X} \pm$ S.D.	33.6 $\bar{X} \pm$ S.D.	Mean $\bar{X} \pm$ S.D.
Unrelated	268	0.172 ± 0.102	0.155 ± 0.082	0.219 ± 0.115	0.182 ± 0.073
Half-sibs	21	0.494 ± 0.095	0.450 ± 0.050	0.605 ± 0.050	0.516 ± 0.049
Full-sibs	145	0.621 ± 0.137	0.622 ± 0.110	0.690 ± 0.103	0.644 ± 0.082
Parent-offspring	123	0.589 ± 0.122	0.616 ± 0.188	0.646 ± 0.122	0.617 ± 0.096

Figures 1a-c: DNA fingerprints of Hae III digested DNA from the polyandrous nest P2 probed with PER, 33.15 and 33.6. Parentage of all 7 nestlings was assigned to female 1 and male 2. Molecular size markers indicate approximate fragment size. Arrow heads indicate bands that are inherited exclusively from the father.

1a



1b



1c

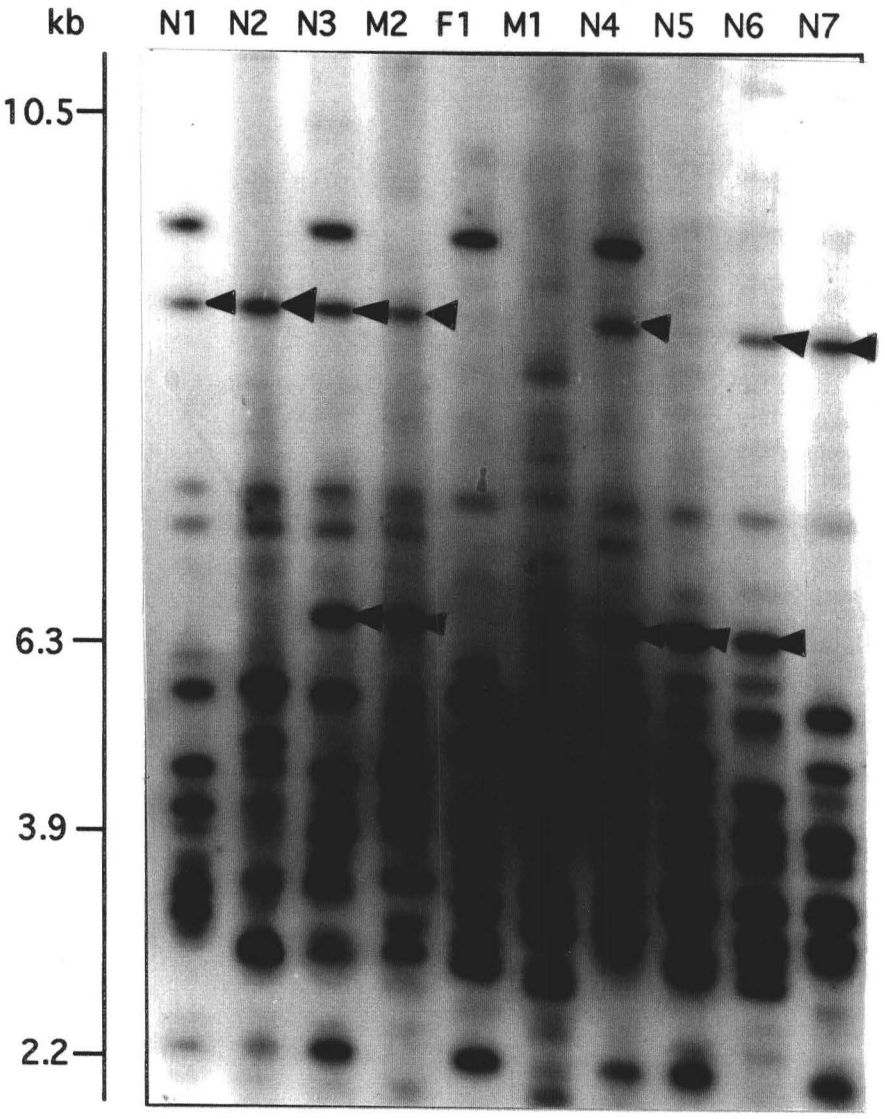
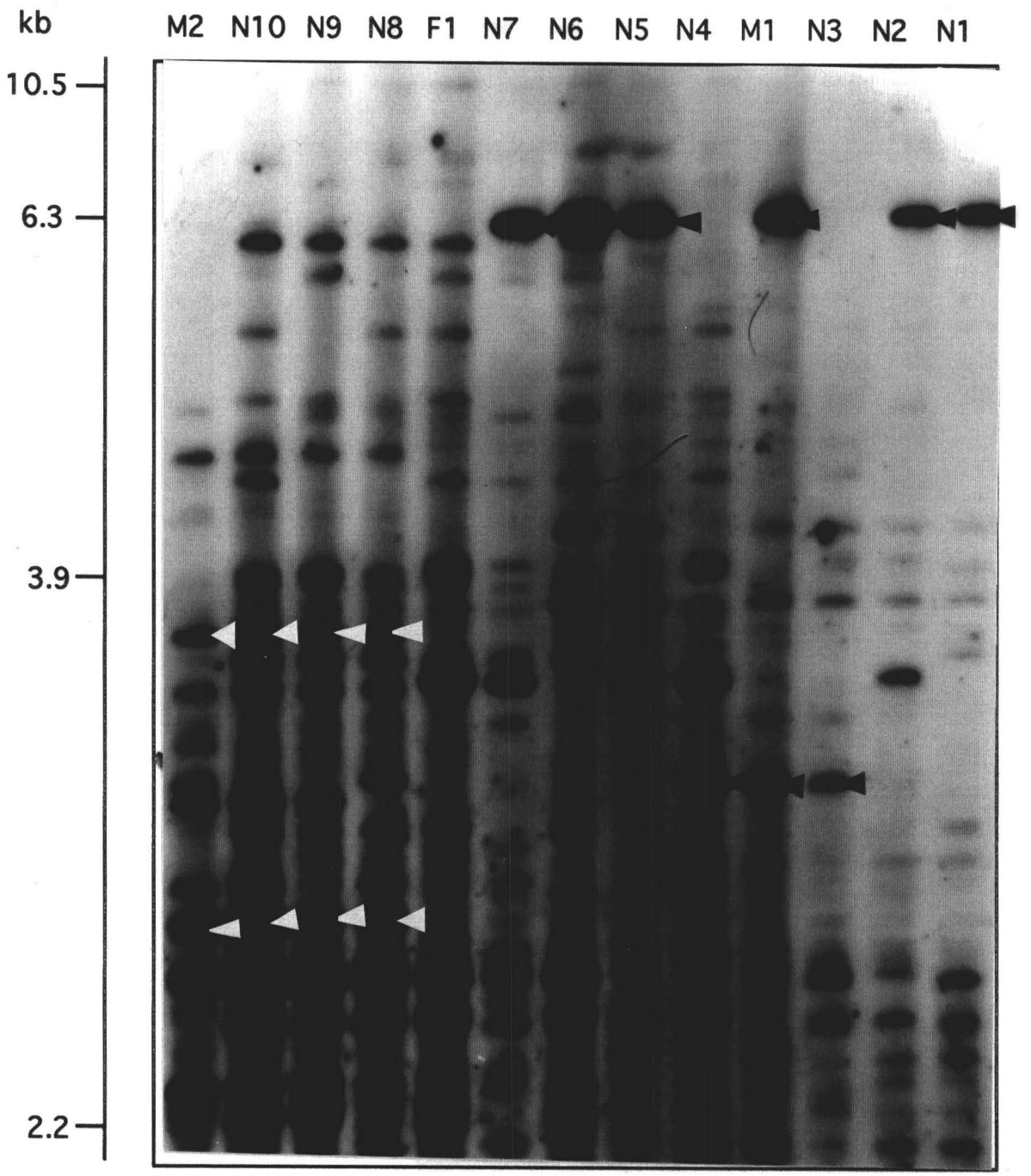


Figure 2: DNA fingerprints of Hae III digested DNA from the double-brood nest #207 probed with PER. Molecular size markers indicate approximate fragment size. White arrow heads indicate bands that are inherited by nestlings in the first brood exclusively from the father (male 1). Black arrow heads indicate bands that are inherited by second brood nestlings from male 2.

2



(To be submitted)

Use of similarity coefficients from DNA fingerprinting to assess relatedness in bushtits (Psaltriparus minimus)

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Abstract

We attempted to estimate relatedness of nestling dyads with similarity coefficients (D-scores) calculated from DNA fingerprints from a population of bushtits. A previous study by Piper and Rabenold (1992) suggested that similarity coefficients would allow assignment of kin relationships to dyads of unknown relatedness. D-scores of known relationships from complete families, those in which both parents and nestlings were sampled, were compared to those derived from families which lacked parental samples. Overlap was present between the confidence intervals of an expected half-sibling distribution and those of unrelated and known full-sibling dyads. The D-scores from the broods lacking parental samples fell in a range overlapping the full-sib distribution predominantly, and somewhat into the half-sib range. Statistical comparisons between the distributions of full-sibs and of dyads unknown relatedness indicated that they were not significantly different, when the distributions were adjusted according to their respective background bandsharing. The results provide strong evidence that there was no incidence of intra-specific brood parasitism (ISBP), and suggest that extra-pair fertilization (EPF) did not occur, to any significant frequency. The utility of using DNA fingerprinting as a means of estimating relatedness is discussed in terms of statistical, methodological and biological factors which can inhibit such analyses.

Introduction

Applications of multilocus DNA fingerprinting, the use of probes to generate individual-specific genetic profiles, have increased dramatically in recent years. Fingerprinting has been used to detect genetic differences in a fairly broad range of applications. Questions ranging from the population level down to the level of related individuals have been addressed using this technique.

Behavioural ecologists have utilized the power of fingerprinting to investigate parentage in a wide variety of species (eg. Jeffreys et al 1985; Wetton et al. 1987; Kovacs 1991). Because the DNA fragments in a fingerprint are inherited in a Mendelian fashion, parentage assignment can be as simple as ensuring that all of the fragments present in an offspring are accounted for by the fingerprints of the putative parents. One advantage in using fingerprinting over RFLPs is the number and level of variability of loci surveyed; fingerprinting surveys many hypervariable loci thereby making the analysis more powerful. The presence of a significant number of unexplained fragments in an offspring's fingerprint implicates an extra-pair fertilization (EPF) or intra-specific brood parasitism (ISBP) event. The discovery of EPFs, ISBPs and other reproductive tactics, in a variety of breeding systems, via molecular genetics has reshaped our understanding of breeding systems.

The similarity coefficient (D) represents the proportion of fragments shared between two fingerprints (Wetton et al. 1987). Theoretically, this measure should provide an indication of genetic relatedness between two individuals (or a dyad). At the finest level of resolution, the D-score could indicate the level of kin relatedness between individuals for which such information is unknown. The limitations of these analyses have been identified by Lynch's (1988) observation that the determination of accurate estimates of relatedness would be severely hampered by the background fragment-sharing in a population (degree to which unrelated individuals share bands). Despite this, a number of studies have attempted to estimate relatedness using D-scores.

There has been a variety of approaches used to estimate relatedness from similarity coefficients. Westneat (1990) calculated 95% confidence intervals from D-scores of known relatedness of indigo buntings (Passerina cyanea). The scores used in this calculation were the mean of three scores from three different probes. Genetic relationships were assigned to dyads of unknown relatedness according to the confidence interval within which the D-score of the dyad fell. The approach used by Quinn et al. (In Press) utilizes a 2X2 Chi square contigency table with adjusted expected frequencies to generate expected frequencies of unrelated pairs. From this they were able to distinguish first and second order from less related dyads.

To date, the most intensive attempt at the use of D-scores to assess relatedness was Piper and Rabenold's (1992) study of stripe-backed wrens (Campylorynchus nuchalis). They used the average of eight D-scores for each dyad derived from using two enzymes, two probes and two scorers. By using the mean of the D-scores they were able to differentiate effectively between first and second-order relatives and unrelated dyads, though only when individuals were run in adjacent lanes were compared, as opposed to comparisons across all lanes. They suggest that in situations where DNA samples are available for offspring only, the use of multiple probes and/or multiple enzymes should provide enough resolving power to distinguish between first and second-order relatives.

Our study used distributions of D-scores from three probings to compare dyads of known relatedness, with dyads of unknown relatedness to gain information on the genetic compositions of broods of bushtits (Psaltiparus minimus) for which parental DNA was not available. This also served as a useful test of Piper and Rabenold's (1992) approach.

Materials and Methods

Study Population

Between 1986 and 1992 the breeding biology of bushtits in the Chiricahua Mountains of southeastern Arizona has been studied. Blood samples from 55 nestlings (from 12 nests) were obtained during the 1988 breeding season, a year of relatively high nesting density. In 1992, a year of lower nesting density, blood samples were collected from 9 complete families, nestlings and adults, consisting of 59 nestlings and 20 adults (from 9 nests) (Bruce, unpubl).

Blood samples of 5-50μl in 1988 and 1992 were collected by brachial venipuncture. The brachial vein was lanced with a 27 guage needle and the blood was collected in a heparinized capillary tube. Blood in EDTA buffer was frozen in 1988, and stored in lysis buffer in 1992. Nestlings were sampled at 12-13 days of age (approximately 2 days before fledging) and immediately returned to the nest.

Molecular Methods

Genomic DNA was extracted from blood stored in lysis buffer (4 M Urea; 0.2 M NaCl: 0.1 M Tris-HCl, pH 8.0; 0.5% n-lauroylsarcosine; 0.01 M CDTA). Between 10 and 25 μl of blood was

mixed in 3.0 ml of lysis buffer. Protein was digested at 37°C using two additions of 100 μl (80 units) of proteinase K. Samples were extracted twice with phenol/chloroform (70:30), once with choloroform and then ethanol precipitated. The DNA was dissolved in 0.2 to 0.6 ml of TNE_2 (0.01 M Tris-HCl, 0.01 M NaCl, 0.002 M EDTA, pH 8.0) and quantified by flourometry and agarose gel electrophoresis.

Approximately 10 μg of DNA was digested for 4-5 h with the restriction enzyme Hae III. The cut DNA was precipitated in 70% ethanol and 0.3 M sodium acetate and redissolved in 20 μl of TNE_2. Four μg of sample DNA was combined with 3 ng of a DNA cocktail of lambda digested with Hind III/EcoR I and BstE II as a control for possible differential mobility between samples (Galbraith et al. 1991), and loaded in a 0.8% agarose gel. Electrophoresis was performed at 1.2-1.5 V/cm for approximately 45 h. The DNA was transferred with 10X SSC (3 M NaCl, 0.3 M sodium citrate, pH 7.0) by Southern blotting to charged polyvinylidene difluoride membrane (Immobilon-N) which was dried and then baked at 80°C for 1-2 h.

Blots were incubated for 2-12 hours at 65°C in prehybridization solution (7% SDS, 0.001 M EDTA, 0.26 M sodium phosphate pH 8.0 and 1% bovine serum albumin; fraction V; Westneat et al. 1988). Minisatellite sequences 33.6 and 33.15 (Jeffreys et al. 1985) and the mouse probe pSP2.5RI (homologous to the Drosophila periodic locus; PER; Georges et al. 1988) were used sequentially as hybridization probes. Probes were labelled by random primer

extension (Pharmacia oligolabelling Kit; >8X10^8 cpm/ug) with ^{32}P-dCTP and hybridizations run overnight at 65°C. Blots were then washed in 2X SSC, 0.1% SDS three times; once for 15 mins. at room temperature, once for 15 mins. at 65°C, and once for 30 mins. at 65°C. X-ray film, with intensifying screens, was exposed to the radioactive blot at -70°C for 1 to 14 days. We stripped blots with 0.4 M NaOH at 42°C for 30 min for repeated probings. Following probing with fingerprint probes, the membranes were probed with lambda, to provide size markers.

The banding patterns of individuals were compared with those run on the same gel. Bands were considered to be the same if their relative intensities were similar (if less than twice the intensity) and if the position of the bands were the same using internal size markers as guides (Galbraith et al. 1991).

Similarity Coefficients

The statistic $\underline{D}=2N_{AB}/(N_A+N_B)$ was calculated where N_{AB} is the number of bands shared by both lanes and N_A and N_B are the number of bands found in lane A or B, respectively (Wetton et al. 1987). The $\underline{D}$-score, which represents the proportion of bands shared between individuals of a dyad, can vary theoretically from zero, when no bands are shared, to one, when all bands are shared.

For each dyad, three $\underline{D}$-scores were calculated, one from each fingerprint probing. The mean of these scores was used in all

comparisons and calculations, similar to the approach taken by Piper and Rabenold (1992). Frequency distributions of dyads of known relatedness (1992 samples) were constructed and 95% confidence intervals calculated according to the formula:

$$95\%\ CI = \bar{X} \pm 1.96\ s$$

$$\text{where } s = \sqrt{\frac{\Sigma\ (X_i - \bar{X})^2}{N-1}}$$

Due to a small sample size (21 dyads) of half-siblings, all sharing the same mother, in 1992, the expected 95% confidence interval was estimated by shifting the interval for full-siblings down according to the expression for observed bandsharing $b = x + r\ (1 - x)$: where x represents the average proportion of bands shared between unrelated individuals; r represents the coefficient of relatedness. D-scores from 1988 dyads (unknown relatedness) were tabulated and a frequency distribution was contructed. To facilitate comparison between the 1988 and 1992 D-score distributions, the data were adjusted down according to the background bandsharing (the proportion of bands shared between unrelated individuals in a population), and then plotted. This was a means of controlling for differences in background bandsharing between the 1988 and 1992 populations. In order to enable direct comparison with Piper and Rabenold (1992), we calculated the standard deviation without accounting for the lack of independence in the data, due to multiple comparisons with the same individual (Lynch, 1990). Means of D-scores are given ± S.D.

Statistical comparisons between 1988 and 1992 distributions were performed using NTSYS (PC version) and Statview512+ (version 1.2, Abacus, Inc. 1988). A Mantel test was used to test for differences between the distributions. This test uses a matrix comparison which is appropriate when the data lack independence due to multiple representation of individuals in dyads. An F-ratio was used to test for differences in variance.

Results

Fingerprints

The three probes used revealed highly variable banding patterns. The DNA fragment size ranges hybridizing to PER, 33.15 and 33.6 were 2.2 - 13, 2.2 - 20 and 2.2 - 16 kb respectively. The extent of duplicated detection of fragments between probes were as follows: 0.22 and 0.16 for PER vs 33.15; 0.21 and 0.18 for PER vs 33.6; and 0.19 and 0.23 for 33.15 vs 33.6. The number of scored fragments detected by the probes PER, 33.15 and 33.6 were $\overline{X}$ = 18.7 ±4.9; $\overline{X}$ = 16.1 ±3.9; and $\overline{X}$ = 13.0 ±4.8 respectively. Figures 1 a-c and 2 a-c illustrate three probings of: 1) a complete family from 1992; parentage was assigned to female 1 and male 2; and 2) of a blot containing the fingerprints of 2 broods from 1988.

D-score Distributions

The mean of D-scores across three probes was used to calculate 95% confidence intervals for full-sibling dyad ($\bar{X}$ = 0.644 ±0.82, n=142) and unrelated dyad ($\bar{X}$ = 0.186 ± 0.073, n=288) distributions. The ranges of the confidence intervals were as follows: full-siblings, 0.289 - 0.631; half-siblings (expected), 0.085 - 0.427; and unrelateds, 0.043 - 0.145. The extent of overlap between the intervals is illustrated in Figure 3a.

The distribution of D-scores from the 1988 dyads are found in Figure 3b. The mean for unrelated dyads was 0.168 (±0.070) and for nestmates (unknown relatedness) 0.599 (±0.076). The 1992 and 1988 D-scores were standardized by deducting the average proportion of bands shared between unrelated individuals (background bandsharing) from each score; 0.184 was subtracted from the 1992 scores and 0.168 from the 1988 scores (Fig 3).

Comparisons between the 1988 and 1992 distributions of unrelated dyads and between the full-sibling (1992) and nestmate (1988) distributions, using the Mantel test which computes a t-value comparison against 1000 permutations. This test indicated a significant difference between distributions (t=2.52, p=0.046) and (t=2.89, p=0.038) respectively. Repeating the analysis on the adjusted distributions showed no difference between the full-sibling and nestmate distributions (t=1.78, p=0.072). No difference

between the variances was detected ($F=1.016$, $p>0.05$) and ($F=1.102$, $p>0.05$) respectively.

Discussion

Similarity coefficients from DNA fingerprints of complete families of bushtits were similar to other outbred bird species. The range of the 95% confidence interval of D-scores calculated for full-sibling dyads can be an informative comparison across species. Using the mean score across three probes, the full-sibling confidence interval range for bushtits was 0.34 compared with that calculated from four D-scores (two probes and two scores) for stripe-backed wrens (0.29). This comparison suggests that the distribution of known full-siblings of bushtits is slightly larger from those derived from a comparable number of stripe-backed wrens D-scores (Piper and Rabenold,1992).

Comparisons between the full-sibling and nestmate distributions were made as a means of estimating the genetic compositions of the 1988 families. Based on the 95% confidence intervals, it is clear that there are no unrelated dyads, resulting from ISBP or communal breeding. Discounting ISBP, which would result in unrelated nestlings, is interesting given that behavioural observations suggest egg dumping may occur (Sloane, 1992).

Statistical comparisons, of the respective distributions between years, indicated significant differences. The comparison

between the unrelated dyads of 1988 and 1992 revealed a difference in bandsharing among unrelateds in the populations, with the 1988 population having a lower background bandsharing than the 1992. We hypothesize that the 1992 birds, which were recolonated the area following a winter in which all of the previous residents died, were derived from the same breeding flock which had a higher degree of relatedness. A significant proportion this population had previously been banded with banding continuing through 1991. In 1992, the lack of any of these birds being observed provided strong evidence that the population had crashed prior to the 1992 breeding season, due to an unusually cold and wet period (Sloane, unpulished data). The difference between the known full-sibling and nestmate distributions is a reflection of the background bandsharing difference, because when the adjusted distributions were compared the difference was lost. Comparison of the adjusted distributions (Figure 3), along with the lack of difference between the variances, suggests that most of the dyads of nestmates from 1988 are full-siblings, but the possibility of a small proportion of half-sibling dyads cannot be ruled out. A lack of half-sibling dyads, would represent conclusive evidence against any incidence of EPF in the 1988 families.

The utility in using DNA fingerprinting data to establish relatedness can be influenced by statistical, methodological and biological factors. Statistically, the lack of independence in the data, arising from repeated D-score comparisons with the same

individual, poses a significant problem (Lynch, 1990). This redundancy can have the effect of biasing the variance. One way of dealing with this problem is to employ appropriate matrix comparison, such as the Mantel test.

Our results serve as a useful comparison to those of Piper and Rabenold (1992) who used multiple probes, enzymes and scorers approach to generate a mean D-score for each dyad. They were able to differentiate between first and second order relatives only when they compared adjacent lanes, which is not feasible when DNA samples are limited and/or many comparisons are required. Here we used internal size markers (Galbraith et al. 1991), which enable comparison across many lanes. Theoretically, the use of internal size markers should alleviate any problems associated with differential mobility which confounds the comparison across many lanes.

Biological differences between species can effect one's ability to discriminate between different degrees of relatedness. If fingerprint fragments do not segregate independantly there can be a decrease in the resolving ability. In an extreme case, minisatellite loci may be located close together on relatively few chromosomes leading to linkage between many fragments. Non-independent segregation can also arise when one allele is represented by multiple bands (Brock and White, 1993). In a species where linkage is common, a distribution of sibling-dyads would broaden, thereby making analysis more difficult.

The general applicability of the approach outlined by Piper and Rabenold (1992) has some limitations. The use of 95% confidence intervals is dependant on the calculation of variance about the mean, which is most often underestimated when the lack of independence in the data is not accounted. Furthermore, in many species, the DNA fragments in a fingerprint may not segregate in a strict Mendelian fashion. Non-independent segregation can severly hamper attempts at estimating relatedness.

With the advent of microsatellite technology, behavioural ecologists now have a molecular tool which can be very useful to track the inheritance of specific loci through offspring. This enables one to determine allele frequencies and greatly increases the ability to discriminate between different degrees of relatedness. Microsatellites can generate abundant and unambiguous genetic data that can be consistently scored (Queller et al 1993). Reanalysis of the DNA from this study would be a very useful comparison between fingerprinting and microsatellites for estimation of kin relatedness.

Acknowledgments

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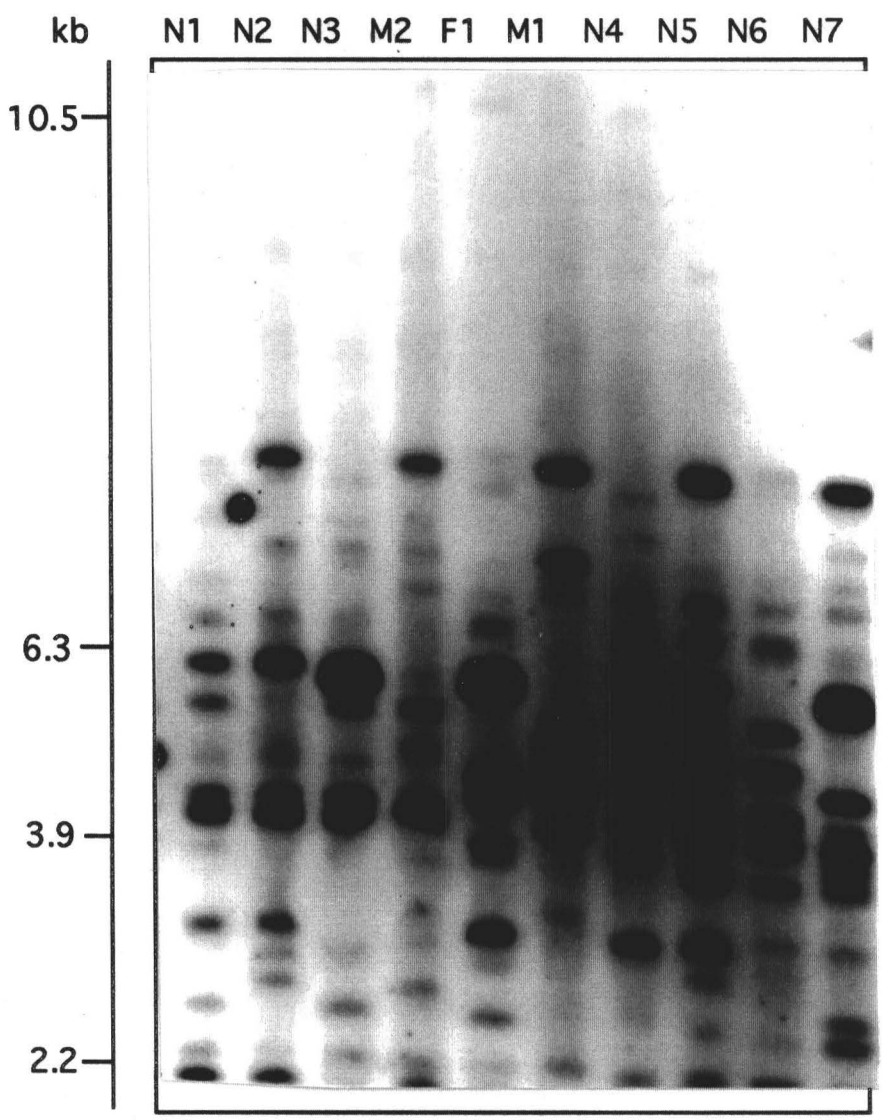
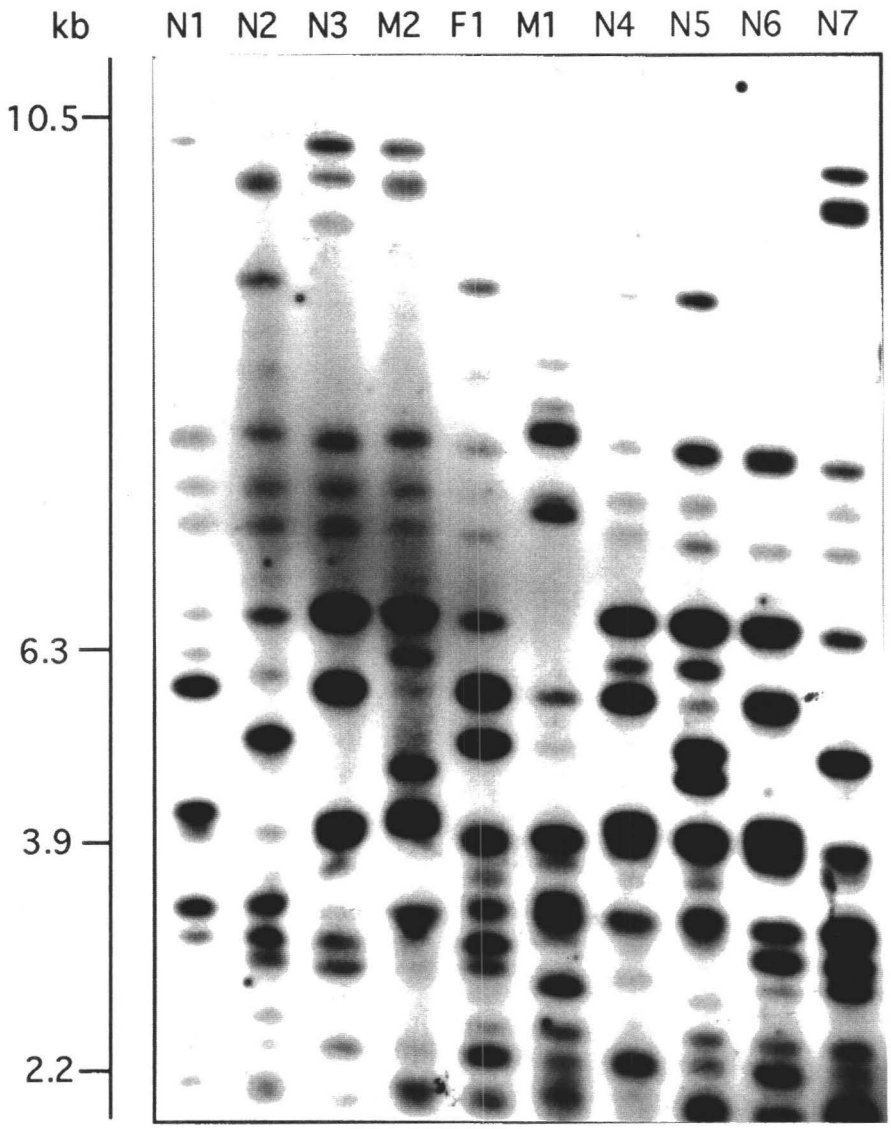
Figures 1a-c: DNA fingerprints of Hae III digested DNA from a polyandrous nest, parented by female 1 and male 2, probed with PER, 33.15 and 33.6. Molecular size markers indicate approximate fragment size.

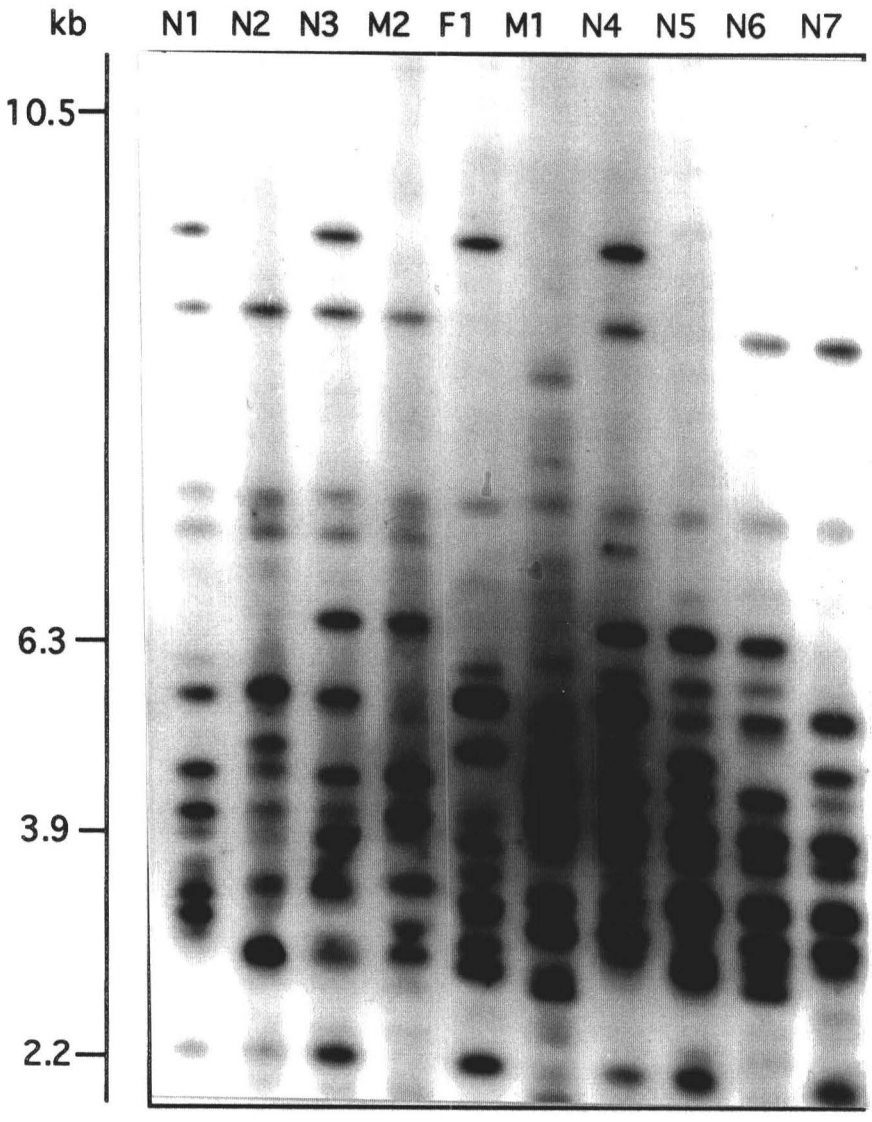
1a



1b

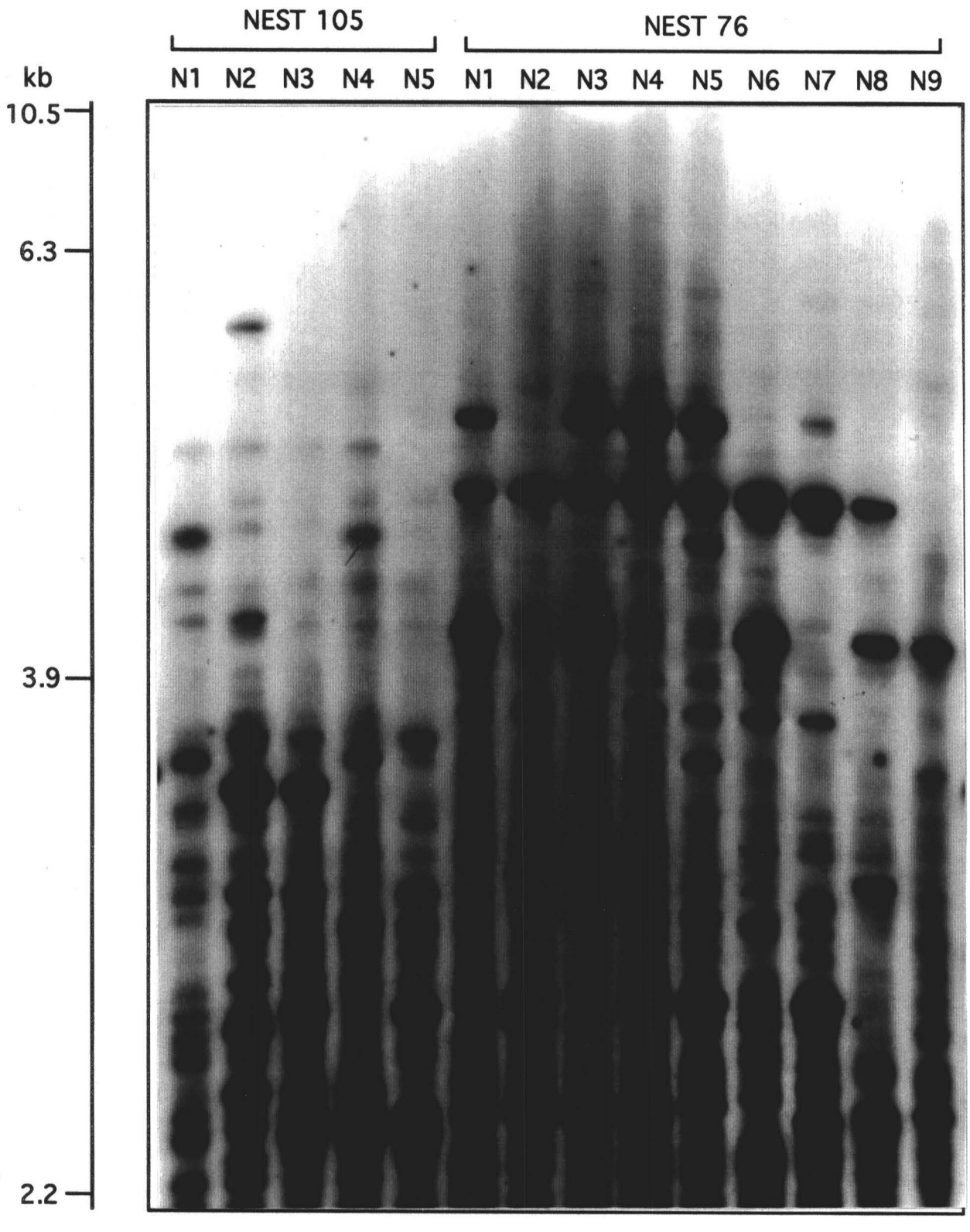


1c

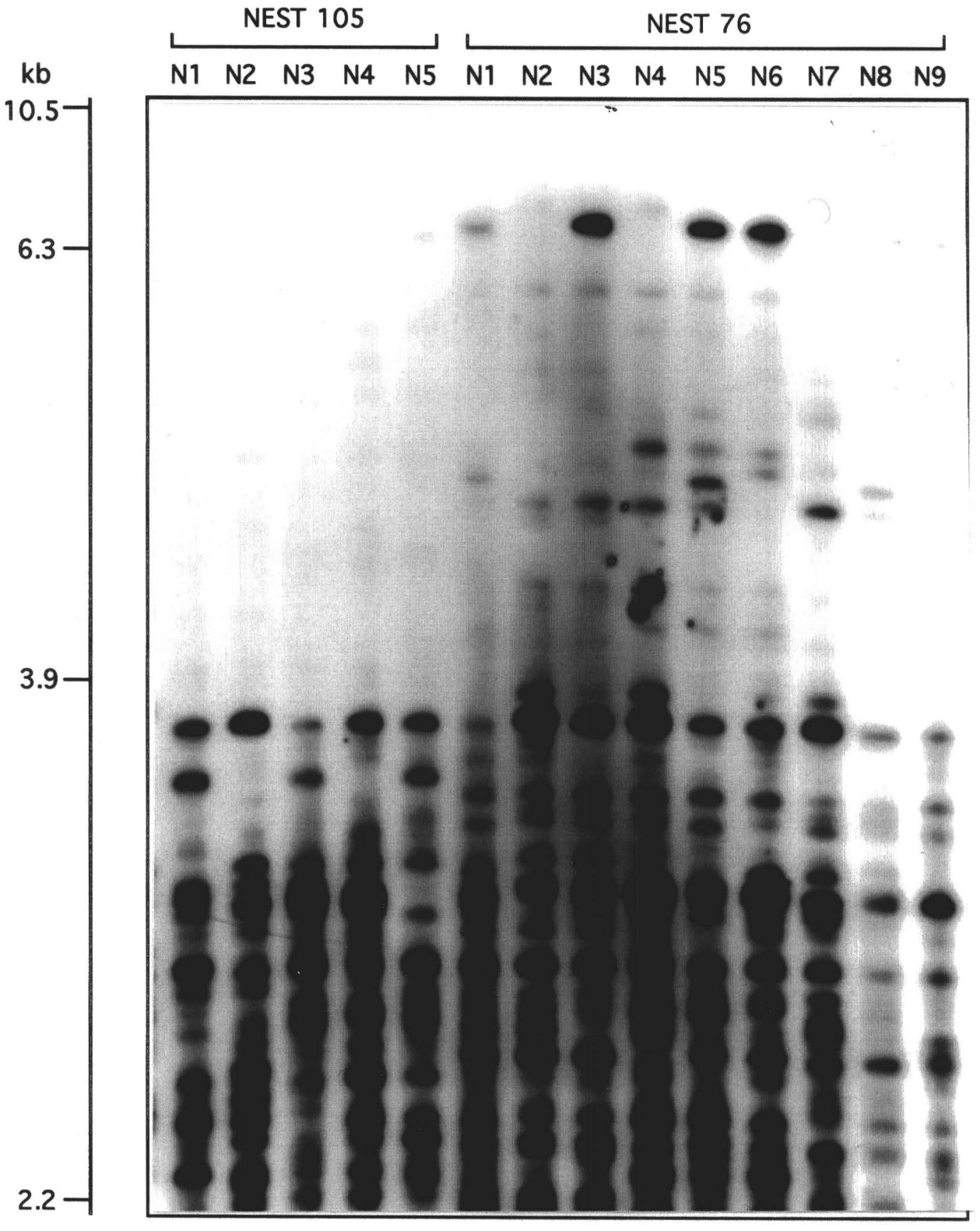


Figures 2a-c: DNA fingerprints of Hae III digested DNA from nestlings of two nests (no parental samples) probed with PER, 33.15 and 33.6. Molecular size markers indicate approximate fragment size.

2a


NEST 105
NEST 76
kb
N1 N2 N3 N4 N5 N1 N2 N3 N4 N5 N6 N7 N8 N9
10.5
6.3
3.9
2.2

2b


NEST 105
NEST 76
kb
N1 N2 N3 N4 N5 N1 N2 N3 N4 N5 N6 N7 N8 N9
10.5
6.3
3.9
2.2

2c

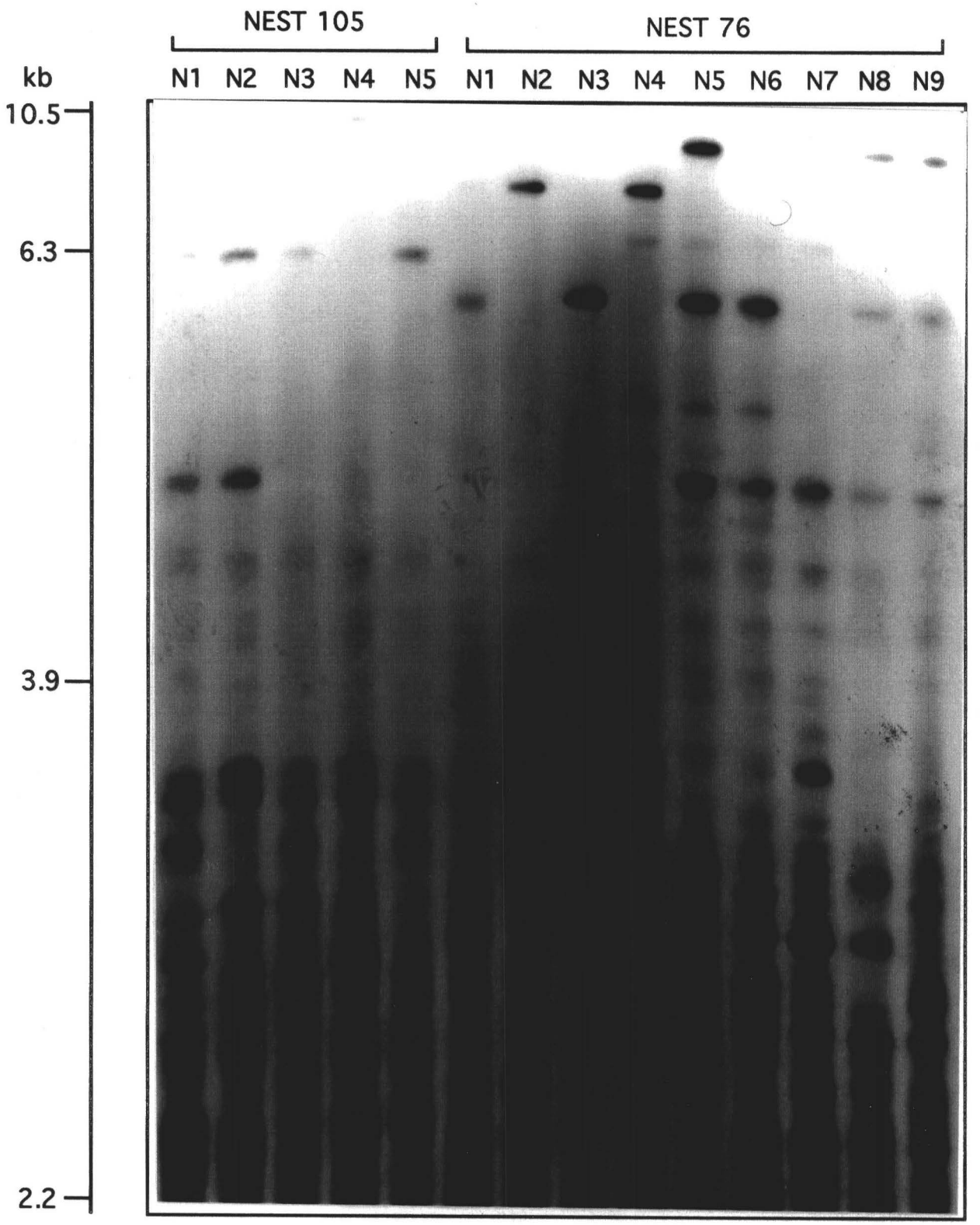
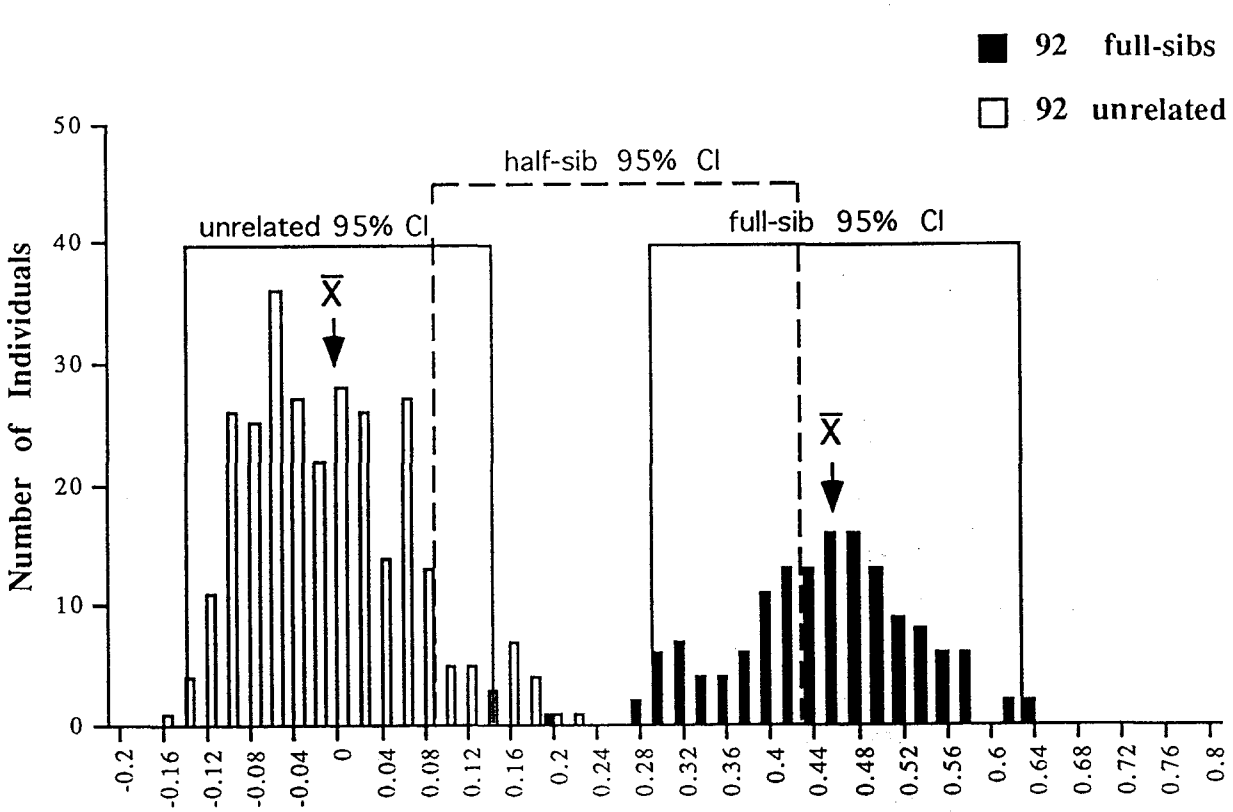

NEST 105
NEST 76
kb
N1 N2 N3 N4 N5 N1 N2 N3 N4 N5 N6 N7 N8 N9
10.5
6.3
3.9
2.2

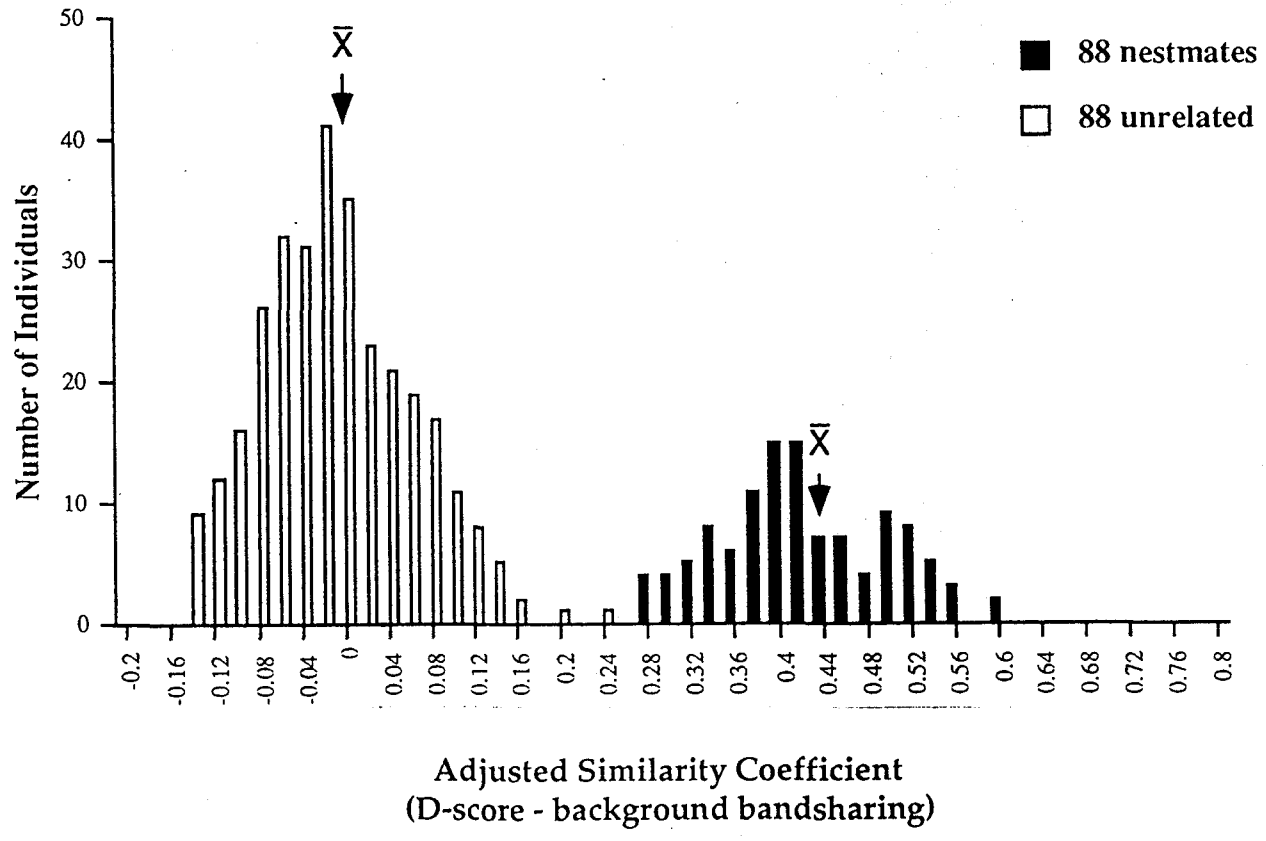
Figure 3a: Frequency distributions of mean D-scores from dyads of known relatedness. Each bar represents an interval of 0.02. Solid lines indicate the 95% confidence intervals calculated for the full-sibling and unrelated dyads, dashed lines indicate the expected 95% confidence intervals for half-siblings.

Figure 3b: Frequency distribution of mean D-scores of dyads from nestlings of unknown relatedness sampled in 1988. Each bar represents an interval of 0.02. Frequency distribution of mean D-scores of dyads from nestlings of unknown relatedness sampled in 1988.

3 a


92 full-sibs
92 unrelated
half-sib 95% CI
unrelated 95% CI
full-sib 95% CI
X̄
X̄
Number of Individuals
50
40
30
20
10
0
-0.2
-0.16
-0.12
-0.08
-0.04
0
0.04
0.08
0.12
0.16
0.2
0.24
0.28
0.32
0.36
0.4
0.44
0.48
0.52
0.56
0.6
0.64
0.68
0.72
0.76
0.8

3 b


88 nestmates
88 unrelated
X̄
X̄
Number of Individuals
50
40
30
20
10
0
-0.2
-0.16
-0.12
-0.08
-0.04
0
0.04
0.08
0.12
0.16
0.2
0.24
0.28
0.32
0.36
0.4
0.44
0.48
0.52
0.56
0.6
0.64
0.68
0.72
0.76
0.8
Adjusted Similarity Coefficient
(D-score - background bandsharing)

Appendix 1

1992 D-SCORES

Nest P2
Nestlings - Pm099 - Pm105
Males - Pm096, Pm098
Female - Pm097

Dyad	PER	33.15	33.6	MEAN
Pm099 X Pm100	.56000	.58333	.83871	.66068
Pm099 X Pm101	.61538	.46154	.75862	.61184
Pm099 X Pm102	.64000	.38462	.78788	.60416
Pm099 X Pm096	.38095	.25000	.44444	.35846
Pm099 X Pm097	.71429	.69231	.57143	.65934
Pm099 X Pm098	.54545	.41667	.66667	.54293
Pm099 X Pm103	.50000	.69231	.66667	.61966
Pm099 X Pm104	.43478	.78571	.69231	.6376
Pm099 X Pm105	.50000	.69231	.70968	.63399
Pm100 X Pm101	.78261	.41667	.80000	.66642
Pm100 X Pm102	.63636	.66667	.82353	.70885
Pm100 X Pm096	.33333	.27273	.42857	.34487
Pm100 X Pm097	.64000	.66667	.62069	.64245
Pm100 X Pm098	.52632	.36364	.64286	.51094
Pm100 X Pm103	.57143	.75000	.64516	.65553
Pm100 X Pm104	.50000	.46154	.59259	.51804
Pm100 X Pm105	.76190	.58333	.75000	.69841
Pm101 X Pm102	.43478	.61538	.68750	.57922
Pm101 X Pm096	.42105	.25000	.38462	.35189
Pm101 X Pm097	.69231	.61538	.59259	.63342
Pm101 X Pm098	.50000	.50000	.53846	.51282
Pm101 X Pm103	.45455	.38462	.62069	.48662
Pm101 X Pm104	.66667	.64286	.64000	.64984
Pm101 X Pm105	.63636	.53846	.60000	.59160
Pm102 X Pm096	.33333	.33333	.40000	.35555
Pm102 X Pm097	.64000	.53846	.58065	.58637
Pm102 X Pm098	.52632	.58333	.73333	.61432
Pm102 X Pm103	.66667	.69231	.84848	.73582
Pm102 X Pm104	.50000	.50000	.62069	.54023
Pm102 X Pm105	.66667	.61538	.88235	.72146
Pm096 X Pm097	.19048	.16667	.16000	.17238

Pm096 X Pm098	.53333	.18182	.41667	.37727
Pm096 X Pm103	.23529	.25000	.29630	.26053
Pm096 X Pm104	.37500	.15385	.34783	.29222
Pm096 X Pm105	.23529	.25000	.35714	.28081
Pm097 X Pm098	.18182	.08333	.16000	.14171
Pm097 X Pm103	.58333	.53846	.57143	.56440
Pm097 X Pm104	.52174	.78571	.41667	.57470
Pm097 X Pm105	.66667	.61538	.68966	.65723
Pm098 X Pm103	.44444	.58333	.66667	.56481
Pm098 X Pm104	.47059	.38462	.69565	.51695
Pm098 X Pm105	.33333	.50000	.57143	.46825
Pm103 X Pm104	.52632	.57143	.69231	.59668
Pm103 X Pm105	.70000	.69231	.70968	.70066
Pm104 X Pm105	.52632	.57143	.59259	.56344

Nest 200
Nestlings - Pm107 - 113
Male - Pm088
Female - Pm107

Pm088 X Pm107	0.13333	0.33333	0.38095	0.28253667
Pm088 X Pm108	0.46154	0.6	0.63636	0.56596667
Pm088 X Pm109	0.42857	0.6	0.58333	0.5373
Pm088 X Pm110	0.47059	0.70968	0.57143	0.5839
Pm088 X Pm111	0.42857	0.68966	0.66667	0.59496667
Pm088 X Pm112	0.57143	0.6	0.6	0.59047667
Pm088 X Pm113	0.30769	0.64286	0.52632	0.49229
Pm107 X Pm108	0.625	0.66667	0.78261	0.69142667
Pm107 X Pm109	0.70588	0.6	0.8	0.70196
Pm107 X Pm110	0.8	0.58065	0.63636	0.67233667
Pm107 X Pm111	0.58824	0.55172	0.8	0.64665333
Pm107 X Pm112	0.58824	0.66667	0.57143	0.60878
Pm107 X Pm113	0.625	0.57143	0.6	0.59881
Pm108 X Pm109	0.26667	0.4	0.76923	0.47863333
Pm108 X Pm110	0.66667	0.58065	0.78261	0.67664333
Pm108 X Pm111	0.53333	0.68966	0.76923	0.66407333
Pm108 X Pm112	0.53333	0.53333	0.63636	0.56767333
Pm108 X Pm113	0.57143	0.64286	0.57143	0.59524
Pm109 X Pm110	0.63158	0.70968	0.64	0.66042
Pm109 X Pm111	0.5	0.41379	0.85714	0.59031
Pm109 X Pm112	0.625	0.53333	0.75	0.63611

Pm109 X Pm113	0.53333	0.57143	0.69565	0.60013667
Pm110 X Pm111	0.63158	0.53333	0.64	0.60163667
Pm110 X Pm112	0.73684	0.64516	0.57143	0.65114333
Pm110 X Pm113	0.55556	0.62069	0.5	0.55875
Pm111 X Pm112	0.625	0.62069	0.83333	0.69300667
Pm111 X Pm113	0.93333	0.44444	0.69565	0.69114
Pm112 X Pm113	0.53333	0.57143	0.63158	0.57878

Nest 201
Nestlings - Pm114-120
Male - Pm085
Female - Pm150

Dyad	PER	33.15	33.6	MEAN
Pm150 X Pm114	0.53333	0.62069	0.82353	0.65918333
Pm150 X Pm115	0.76923	0.66667	0.8125	0.74946667
Pm150 X Pm116	0.61538	0.68966	0.85714	0.61279
Pm150 X Pm117	0.76923	0.68966	0.51613	0.77201
Pm150 X Pm118	0.8	0.64286	0.70588	0.76666667
Pm150 X Pm119	0.61538	0.73333	0.5876	0.62161333
Pm114 X Pm115	0.77778	0.70968	0.82353	0.77033
Pm114 X Pm116	0.77778	0.66667	0.81081	0.66898333
Pm114 X Pm117	0.77778	0.66667	0.54545	0.75175333
Pm114 X Pm118	0.53333	0.68966	0.77778	0.67793333
Pm114 X Pm119	0.77778	0.70968	0.13333	0.67763667
Pm115 X Pm116	0.875	0.58065	0.8	0.68521667
Pm115 X Pm117	0.875	0.77419	0.58065	0.81639667
Pm115 X Pm118	0.76923	0.66667	0.64706	0.7453
Pm115 X Pm119	0.875	0.75	0.28571	0.73521667
Pm116 X Pm117	0.875	0.6	0.64706	0.69368667
Pm116 X Pm118	0.76923	0.68966	0.86486	0.68831667
Pm116 X Pm119	0.875	0.70968	0.3871	0.82707667
Pm117 X Pm118	0.76923	0.62069	0.72727	0.74401
Pm117 X Pm119	0.75	0.58065	0.44444	0.65923667
Pm118 X Pm119	0.76923	0.66667	0.33333	0.69432
Pm150 X Pm120	0.64286	0.64286	0.70588	0.678753
Pm114 X Pm120	0.55172	0.55172	0.77778	0.688374
Pm115 X Pm120	0.6	0.6	0.64706	0.6234856
Pm116 X Pm120	0.55172	0.55172	0.625	0.58674
Pm117 X Pm120	0.62069	0.62069	0.75676	0.68655345

Nest P3
Nestlings - Pm151-154
Male - Pm155
Female - Pm156

Dyad	PER	33.15	33.6	MEAN
Pm155 X Pm151	0.73171	0.74576	0.57143	0.68296667
Pm155 X Pm152	0.68966	0.7037	0.59459	0.66265
Pm155 X Pm153	0.6	0.74074	0.62857	0.65643667
Pm155 X Pm154	0.6	0.59259	0.65	0.61419667
Pm155 X Pm156	0.22222	0.31373	0.24242	0.25945667
Pm151 X Pm152	0.63158	0.68852	0.63158	0.65056
Pm151 X Pm153	0.5641	0.68852	0.66667	0.63976333
Pm151 X Pm154	0.73469	0.62295	0.78049	0.71271
Pm151 X Pm156	0.57778	0.58621	0.70588	0.62329
Pm152 X Pm153	0.59259	0.67857	0.78947	0.68687667
Pm152 X Pm154	0.37838	0.64286	0.65116	0.55746667
Pm152 X Pm156	0.36364	0.64151	0.72222	0.57579
Pm153 X Pm154	0.63158	0.60714	0.63415	0.62429
Pm153 X Pm156	0.52941	0.56604	0.64706	0.58083667
Pm154 X Pm156	0.72727	0.60377	0.66667	0.66590333

Nest 203
Nestlings - Pm143-149
Male - Pm079
Female - Pm080

Dyad	PER	33.15	33.6	MEAN
Pm079 X Pm143	0.55556	0.5	0.66667	0.57407667
Pm079 X Pm144	0.7	0.51064	0.73684	0.64916
Pm079 X Pm145	0.5641	0.60465	0.78947	0.65274
Pm079 X Pm146	0.45714	0.63636	0.62857	0.57402333
Pm079 X Pm147	0.61538	0.53333	0.72222	0.62364333
Pm079 X Pm148	0.7907	0.72	0.52632	0.67900667
Pm079 X Pm149	0.48649	0.47619	0.64865	0.53711
Pm079 X Pm080	0.3	0.14286	0.29412	0.24566
Pm143 X Pm144	0.52941	0.66667	0.66667	0.62091667
Pm143 X Pm145	0.48485	0.34286	0.61111	0.47960667
Pm143 X Pm146	0.62069	0.33333	0.66667	0.54023
Pm143 X Pm147	0.30303	0.64865	0.52941	0.49369667
Pm143 X Pm148	0.54054	0.52381	0.55556	0.53997
Pm143 X Pm149	0.58065	0.52941	0.51429	0.54145

Pm143 X Pm080	0.64706	0.47059	0.5625	0.56005
Pm144 X Pm145	0.43243	0.57143	0.73684	0.58023333
Pm144 X Pm146	0.48485	0.55814	0.51429	0.51909333
Pm144 X Pm147	0.54054	0.68182	0.72222	0.64819333
Pm144 X Pm148	0.58537	0.69388	0.63158	0.63694333
Pm144 X Pm149	0.51429	0.43902	0.59459	0.51596667
Pm144 X Pm080	0.52632	0.63415	0.58824	0.58290333
Pm145 X Pm146	0.4375	0.51282	0.68571	0.54534333
Pm145 X Pm147	0.72222	0.45	0.61111	0.59444333
Pm145 X Pm148	0.65	0.71111	0.57895	0.64668667
Pm145 X Pm149	0.70588	0.43243	0.7027	0.61367
Pm145 X Pm080	0.54054	0.48649	0.52941	0.51881333
Pm146 X Pm147	0.3125	0.53659	0.66667	0.50525333
Pm146 X Pm148	0.33333	0.52174	0.62857	0.49454667
Pm146 X Pm149	0.4	0.47368	0.58824	0.48730667
Pm146 X Pm080	0.60606	0.47368	0.64516	0.57496667
Pm147 X Pm148	0.7	0.6383	0.61111	0.64980333
Pm147 X Pm149	0.64706	0.5641	0.68571	0.63229
Pm147 X Pm080	0.54054	0.61538	0.5625	0.57280667
Pm148 X Pm149	0.63158	0.40909	0.59459	0.54508667
Pm148 X Pm080	0.4878	0.5	0.70588	0.56456
Pm149 X Pm080	0.62857	0.61111	0.54545	0.59504333

Nest P1
Nestlings - Pm091-095
Male - Pm090
Female - Pm106

Dyad	PER	33.15	33.6	MEAN
Pm090 X Pm091	0.72727	0.72727	0.75	0.73484667
Pm090 X Pm092	0.53333	0.65116	0.61538	0.59995667
Pm090 X Pm093	0.74286	0.65217	0.65	0.68167667
Pm090 X Pm094	0.37037	0.61905	0.57143	0.52028333
Pm090 X Pm095	0.6	0.62222	0.66667	0.62963
Pm090 X Pm106	0.27586	0.4186	0.35294	0.34913333
Pm091 X Pm092	0.66667	0.5641	0.60606	0.61227667
Pm091 X Pm093	0.8125	0.66667	0.70588	0.72835
Pm091 X Pm094	0.5	0.63158	0.62069	0.58409
Pm091 X Pm095	0.59259	0.63415	0.54545	0.59073
Pm091 X Pm106	0.53846	0.51282	0.5	0.51709333
Pm092 X Pm093	0.55172	0.68293	0.60606	0.61357

Pm092 X Pm094	0.28571	0.48649	0.42857	0..40026
Pm092 X Pm095	0.5	0.4	0.5625	0..4875
Pm092 X Pm106	0.52174	0.57895	0.51852	0.5397
Pm093 X Pm094	0.46154	0.55	0.48276	0.4981
Pm093 X Pm095	0.68966	0.65116	0.54545	0.62867
Pm093 X Pm106	0.57143	0.58537	0.57143	0.57608
Pm094 X Pm095	0.47619	0.5641	0.5	0.51343
Pm094 X Pm106	0.6	0.64865	0.6087	0.619117
Pm095 X Pm106	0.52174	0.65	0.59259	0.58811

Nest 204
Nestlings - Pm130-135
Male - Pm083
Female - Pm084

Dyad	PER	33.15	33.6	MEAN
Pm083 X Pm130	0.44444	0.72222	0.7027	0.62312
Pm083 X Pm131	0.41379	0.58824	0.47059	0.49087
Pm083 X Pm132	0.5	0.66667	0.77273	0.6465
Pm083 X Pm133	0.4	0.66667	0.65	0.572222
Pm083 X Pm134	0.45161	0.68293	0.55556	0.5633667
Pm083 X Pm135	0.58065	0.60606	0.66667	0.6177933
Pm083 X Pm084	0.17647	0.45	0.28571	0.30406
Pm130 X Pm131	0.58824	0.52632	0.42424	0.51293
Pm130 X Pm132	0.34483	0.7907	0.7907	0.64207667
Pm130 X Pm133	0.4	0.82609	0.61538	0.61382333
Pm130 X Pm134	0.55556	0.62222	0.51429	0.56402333
Pm130 X Pm135	0.66667	0.54054	0.57895	0.59538667
Pm130 X Pm084	0.61538	0.63636	0.52941	0.59371667
Pm131 X Pm132	0.3871	0.68293	0.5	0.52334
Pm131 X Pm133	0.54054	0.63636	0.66667	0.6145233
Pm131 X Pm134	0.68421	0.65116	0.6875	0.67429
Pm131 X Pm135	0.52632	0.68571	0.62857	0.613533
Pm131 X Pm084	0.63415	0.7619	0.64516	0.6804033
Pm132 X Pm133	0.4375	0.77551	0.78261	0.6652066
Pm132 X Pm134	0.60606	0.75	0.61905	0.65837
Pm132 X Pm135	0.48485	0.65	0.71111	0.61532
Pm132 X Pm084	0.55556	0.76596	0.58537	0.63563
Pm133 X Pm134	0.76923	0.7451	0.73684	0.75039
Pm133 X Pm135	0.61538	0.55814	0.78049	0.65133667
Pm133 X Pm084	0.71429	0.8	0.7027	0.73899667

Pm134 X Pm135	0.65	0.61905	0.64865	0.6392333
Pm134 X Pm084	0.74419	0.77551	0.66667	0.72879
Pm135 X Pm084	0.65116	0.63415	0.61111	0.63214

Nest 207 a & b
Nestlings - Pm121-127 (first brood) Pm157-159 (second brood)
Males - Pm089, Pm129
Female - Pm128

Dyad	PER	33.15	33.6	MEAN
Pm121 X Pm122	0.70968	0.70588	0.60377	0.67311
Pm121 X Pm123	0.66667	0.8	0.84615	0.77094
Pm121 X Pm089	0.59259	0.61224	0.64	0.61494333
Pm121 X Pm124	0.60606	0.7451	0.84615	0.73243667
Pm121 X Pm125	0.68571	0.69388	0.7037	0.69443
Pm121 X Pm126	0.77778	0.78571	0.74074	0.76807667
Pm121 X Pm127	0.4	0.75	0.71698	0.62232667
Pm121 X Pm128	0.64706	0.73913	0.74074	0.70897667
Pm121 X Pm157	0.55556	0.66667	0.48148	0.56790333
Pm121 X Pm158	0.54054	0.65385	0.49123	0.56187333
Pm121 X Pm159	0.5	0.58824	0.42308	0.50377333
Pm121 X Pm129	0.13793	0.56	0.30189	0.33327333
Pm122 X Pm123	0.66667	0.57143	0.53061	0.58957
Pm122 X Pm089	0.66667	0.66667	0.68085	0.67139667
Pm122 X Pm124	0.6	0.72	0.44898	0.58966
Pm122 X Pm125	0.875	0.70833	0.7451	0.77614333
Pm122 X Pm126	0.54545	0.72727	0.70588	0.65953333
Pm122 X Pm127	0.59259	0.69091	0.68	0.6545
Pm122 X Pm128	0.51613	0.62222	0.62745	0.5886
Pm122 X Pm157	0.36364	0.51064	0.35294	0.40907333
Pm122 X Pm158	0.35294	0.58824	0.40741	0.44953
Pm122 X Pm159	0.30303	0.48	0.44898	0.41067
Pm122 X Pm129	0.23077	0.40816	0.28	0.30631
Pm123 X Pm089	0.61538	0.55319	0.65217	0.60691333
Pm123 X Pm124	0.8125	0.73469	0.875	0.80739667
Pm123 X Pm125	0.76471	0.59574	0.64	0.66681667
Pm123 X Pm126	0.68571	0.81481	0.64	0.71350667
Pm123 X Pm127	0.55172	0.81481	0.65306	0.67319667
Pm123 X Pm128	0.60606	0.68182	0.64	0.64262667
Pm123 X Pm157	0.45714	0.6087	0.4	0.48861333
Pm123 X Pm158	0.38889	0.64	0.45283	0.49390667

Pm123 X Pm159	0.4	0.65306	0.375	0.47602
Pm123 X Pm129	0.21429	0.54167	0.2449	0.33362
Pm089 X Pm124	0.38462	0.625	0.6087	0.53944
Pm089 X Pm125	0.57143	0.56522	0.66667	0.60110667
Pm089 X Pm126	0.48276	0.67925	0.70833	0.62344667
Pm089 X Pm127	0.34783	0.71698	0.6383	0.56770333
Pm089 X Pm128	0.14815	0.27907	0.41667	0.28129667
Pm089 X Pm157	0.13793	0.31111	0.375	0.27468
Pm089 X Pm158	0.13333	0.32653	0.35294	0.27093333
Pm089 X Pm159	0.06897	0.29167	0.3913	0.25064667
Pm089 X Pm129	0.18182	0.38298	0.21277	0.25919
Pm124 X Pm125	0.70588	0.625	0.6	0.64362667
Pm124 X Pm126	0.62857	0.76364	0.6	0.66407
Pm124 X Pm127	0.68966	0.8	0.61224	0.70063333
Pm124 X Pm128	0.78788	0.62222	0.68	0.6967
Pm124 X Pm157	0.62857	0.59574	0.44	0.55477
Pm124 X Pm158	0.55556	0.62745	0.49057	0.55786
Pm124 X Pm159	0.57143	0.56	0.41667	0.51603333
Pm124 X Pm129	0.28571	0.53061	0.28571	0.36734333
Pm125 X Pm126	0.7027	0.71698	0.88462	0.7681
Pm125 X Pm127	0.70968	0.71698	0.66667	0.69777667
Pm125 X Pm128	0.68571	0.74419	0.76923	0.73304333
Pm125 X Pm157	0.48649	0.66667	0.42308	0.52541333
Pm125 X Pm158	0.47368	0.65306	0.50909	0.54527667
Pm125 X Pm159	0.48649	0.54167	0.52	0.51605333
Pm125 X Pm129	0.26667	0.55319	0.31373	0.37786333
Pm126 X Pm127	0.6875	0.9	0.7451	0.77753333
Pm126 X Pm128	0.77778	0.72	0.69231	0.73003
Pm126 X Pm157	0.57895	0.61538	0.38462	0.52631667
Pm126 X Pm158	0.66667	0.60714	0.50909	0.5943
Pm126 X Pm159	0.57895	0.58182	0.52	0.56025667
Pm126 X Pm129	0.19355	0.51852	0.35294	0.35500333
Pm127 X Pm128	0.73333	0.68	0.70588	0.70640333
Pm127 X Pm157	0.5	0.61538	0.47059	0.52865667
Pm127 X Pm158	0.54545	0.64286	0.44444	0.54425
Pm127 X Pm159	0.4375	0.61818	0.4898	0.51516
Pm127 X Pm129	0.32	0.55556	0.36	0.41185333
Pm128 X Pm157	0.72222	0.80952	0.57692	0.70288667
Pm128 X Pm158	0.75676	0.73913	0.58182	0.69257
Pm128 X Pm159	0.72222	0.71111	0.6	0.67777667

Pm128 X Pm129	0.27586	0.54545	0.31373	0.37834667
Pm157 X Pm158	0.82051	0.83333	0.61818	0.75734
Pm157 X Pm159	0.78947	0.80851	0.68	0.75932667
Pm157 X Pm129	0.64516	0.73913	0.66667	0.68365333
Pm158 X Pm159	0.82051	0.82353	0.49057	0.71153667
Pm158 X Pm129	0.625	0.84	0.74074	0.73524667
Pm159 X Pm129	0.58065	0.81633	0.65306	0.68334667

Nest 206
Nestlings - Pm136-141
Male - Pm082
Female - Pm142

Dyad PER	33.15	33.15	MEAN	
Pm082 X Pm136	0.57143	0.64865	0.66667	0.62891667
Pm082 X Pm137	0.51852	0.74286	0.73171	0.66436333
Pm082 X Pm138	0.55556	0.65	0.76923	0.65826333
Pm082 X Pm139	0.6	0.68421	0.71795	0.66738667
Pm082 X Pm140	0.5625	0.73684	0.75	0.68311333
Pm082 X Pm141	0.58824	0.47368	0.71795	0.59329
Pm082 X Pm142	0.29412	0.36842	0.51282	0.39178667
Pm136 X Pm137	0.51613	0.73684	0.86364	0.70553667
Pm136 X Pm138	0.65	0.65116	0.71429	0.67181667
Pm136 X Pm139	0.58824	0.53659	0.71429	0.61304
Pm136 X Pm140	0.66667	0.68293	0.7907	0.71343333
Pm136 X Pm141	0.73684	0.68293	0.7619	0.72722333
Pm136 X Pm142	0.63158	0.68293	0.85714	0.72388333
Pm137 X Pm138	0.5641	0.63415	0.77273	0.65699333
Pm137 X Pm139	0.60606	0.71795	0.81818	0.71406333
Pm137 X Pm140	0.57143	0.76923	0.84444	0.72836667
Pm137 X Pm141	0.59459	0.51282	0.77273	0.62671333
Pm137 X Pm142	0.64865	0.61538	0.77273	0.67892
Pm138 X Pm139	0.71429	0.63636	0.71429	0.6883133
Pm138 X Pm140	0.90909	0.77273	0.83721	0.83967667
Pm138 X Pm141	0.73913	0.77273	0.71429	0.74205
Pm138 X Pm142	0.82609	0.77273	0.66667	0.75516333
Pm139 X Pm140	0.68421	0.7619	0.74419	0.7301
Pm139 X Pm141	0.75	0.52381	0.71429	0.6627
Pm139 X Pm142	0.65	0.61905	0.71429	0.6611133
Pm140 X Pm141	0.7619	0.61905	0.65116	0.67737
Pm140 X Pm142	0.7619	0.61905	0.7907	0.7238833

Pm141 X Pm142	0.72727	0.80952	0.71429	0.75036

Appendix 2

1988 D-scores

Nest - 94
Nestlings - Pm213-215

Dyad	PER	33.15	33.6	MEAN
Pm213 X Pm214	0.7	0.61538	0.73684	0.68407333
Pm213 X Pm215	0.66667	0.61538	0.72	0.66735
Pm214 X Pm215	0.76923	0.8	0.66667	0.7453

Nest - 104
Nestlings - Pm216-219

Pm216 X Pm217	0.46667	0.5	0.4	0.45555667
Pm216 X Pm218	0.55172	0.4	0.63636	0.52936
Pm216 X Pm219	0.53333	0.5625	0.48	0.52527667
Pm217 X Pm218	0.48649	0.61111	0.64516	0.58092
Pm217 X Pm219	0.68421	0.73684	0.70588	0.70897667
Pm218 X Pm219	0.54054	0.75	0.64516	0.64523333

Nest - 88
Nestlings - Pm209-212

Pm209 X Pm210	0.62069	0.59091	0.68421	0.6319366
Pm209 X Pm212	0.53333	0.65217	0.66667	0.61739
Pm210 X Pm212	0.81081	0.625	0.8	0.74527

Nest - 112
Nestlings - Pm226-228

Pm226 X Pm227	0.48276	0.61538	0.32432	0.47415
Pm226 X Pm228	0.63158	0.85714	0.76364	0.7507866
Pm227 X Pm228	0.68354	0.75435	0.65653	0.69814

Nest - 87
Nestlings - Pm173-176

Pm173 X Pm174	0.51852	0.57143	0.57143	0.55379333
Pm173 X Pm175	0.56	0.77419	0.72222	0.70279333
Pm173 X Pm176	0.68966	0.68571	0.59459	0.68702667
Pm174 X Pm175	0.66667	0.70968	0.6875	0.69534333
Pm174 X Pm176	0.64706	0.62857	0.54545	0.63473333
Pm175 X Pm176	0.75	0.73684	0.78049	0.74122667

Nest - 92
Nestlings - Pm177-181

Dyad	PER	33.15	33.6	MEAN
Pm177 X Pm178	0.58824	0.55319	0.73077	0.56487333

Pm177 X Pm179	0.8	0.58182	0.78689	0.65454667
Pm177 X Pm180	0.58824	0.61224	0.69231	0.60424
Pm177 X Pm181	0.64865	0.59574	0.72727	0.61337667
Pm178 X Pm179	0.58824	0.74074	0.61818	0.68990667
Pm178 X Pm180	0.42857	0.625	0.73913	0.5975666
Pm178 X Pm181	0.51613	0.6087	0.65306	0.59263
Pm179 X Pm180	0.47059	0.60714	0.61818	0.5653033
Pm179 X Pm181	0.64865	0.55556	0.75862	0.6542766
Pm180 X Pm181	0.70968	0.70833	0.61224	0.67675

Nest - 76 a & b

Nestlings - Pm195-197 (a brood), Pm198-201,208 (b brood)

Pm193 X Pm195	0.4	0.58824	0.47059	0.48627667
Pm193 X Pm196	0.46154	0.47059	0.51282	0.48165
Pm193 X Pm197	0.37037	0.68293	0.7	0.58443333
Pm193 X Pm198	0.53846	0.52941	0.59459	0.55415333
Pm193 X Pm199	0.33333	0.52941	0.63158	0.49810667
Pm193 X Pm200	0.4	0.51429	0.61111	0.50846667
Pm193 X Pm201	0.37037	0.58537	0.48649	0.48074333
Pm193 X Pm208	0.36364	0.64516	0.375	0.46126667
Pm195 X Pm196	0.55172	0.6	0.57143	0.57438333
Pm195 X Pm197	0.6	0.6383	0.5	0.57943333
Pm195 X Pm198	0.41379	0.75	0.48485	0.54954667
Pm195 X Pm199	0.51852	0.5	0.41176	0.47676
Pm195 X Pm200	0.78571	0.78049	0.5625	0.70956667
Pm195 X Pm201	0.66667	0.6383	0.48485	0.59660667
Pm195 X Pm208	0.48	0.7027	0.35714	0.51328
Pm196 X Pm197	0.64516	0.6383	0.78049	0.68798333
Pm196 X Pm198	0.6	0.55	0.73684	0.62894667
Pm196 X Pm199	0.64286	0.5	0.66667	0.60317667
Pm196 X Pm200	0.48276	0.73171	0.48649	0.56698667
Pm196 X Pm201	0.70968	0.68085	0.63158	0.67403667
Pm196 X Pm208	0.69231	0.59459	0.36364	0.55018
Pm197 X Pm198	0.51613	0.76596	0.76923	0.68377333
Pm197 X Pm199	0.62069	0.7234	0.7	0.68136333
Pm197 X Pm200	0.6	0.66667	0.57895	0.61520667
Pm197 X Pm201	0.625	0.77778	0.46154	0.62144
Pm197 X Pm208	0.59259	0.59091	0.41176	0.53175333
Pm198 X Pm199	0.57143	0.7	0.59459	0.62200667
Pm198 X Pm200	0.41379	0.68293	0.45714	0.51795333
Pm198 X Pm201	0.58065	0.59574	0.38889	0.52176

Pm198 X Pm208	0.38462	0.59459	0.3871	0.45543667
Pm199 X Pm200	0.66667	0.53659	0.44444	0.54923333
Pm199 X Pm201	0.68966	0.7234	0.64865	0.68723667
Pm199 X Pm208	0.75	0.54054	0.5	0.59684667
Pm200 X Pm201	0.73333	0.70833	0.62857	0.69007667
Pm200 X Pm208	0.56	0.63158	0.6	0.59719333
Pm201 X Pm208	0.59259	0.59091	0.58065	0.58805

Nest - 105

Nestlings - Pm221-225

Dyad	PER	33.15	33.6	MEAN
Pm221 X Pm222	0.73333	0.65	0.83871	0.74068
Pm221 X Pm223	0.66667	0.60606	0.81481	0.69584667
Pm221 X Pm224	0.60606	0.58537	0.77419	0.65520667
Pm221 X Pm225	0.64	0.68571	0.53333	0.61968
Pm222 X Pm223	0.51852	0.59459	0.64286	0.58532333
Pm222 X Pm224	0.60606	0.75556	0.8125	0.72470667
Pm222 X Pm225	0.56	0.5641	0.51613	0.54674333
Pm223 X Pm224	0.6	0.47368	0.71429	0.59599
Pm223 X Pm225	0.45455	0.5	0.51852	0.49102333
Pm224 X Pm225	0.42857	0.55	0.64516	0.54124333

Nest - 90 a & b

Nestlings - Pm206,207 (**a** brood), Pm165-170 (**b** brood)

Pm206 X Pm207	0.61538	0.5641	0.37037	0.51661667
Pm206 X Pm165	0.82759	0.58065	0.52174	0.64332667
Pm206 X Pm166	0.37037	0.60606	0.5	0.49214333
Pm206 X Pm167	0.56	0.47059	0.57143	0.53400667
Pm206 X Pm168	0.43478	0.35294	0.26087	0.34953
Pm206 X Pm169	0.41667	0.48485	0.44444	0.44865333
Pm206 X Pm170	0.26087	0.46667	0.42105	0.38286333
Pm207 X Pm165	0.64516	0.68182	0.53333	0.62010333
Pm207 X Pm166	0.48276	0.65217	0.64516	0.59336333
Pm207 X Pm167	0.51852	0.76596	0.64286	0.64244667
Pm207 X Pm168	0.48	0.7234	0.73333	0.64557667
Pm207 X Pm169	0.61538	0.69565	0.4	0.57034333
Pm207 X Pm170	0.56	0.60465	0.46154	0.54206333
Pm165 X Pm166	0.5	0.78947	0.51852	0.60266333
Pm165 X Pm167	0.66667	0.76923	0.33333	0.58974333
Pm165 X Pm168	0.5	0.66667	0.46154	0.54273667
Pm165 X Pm169	0.48276	0.68421	0.57143	0.57946667
Pm165 X Pm170	0.28571	0.57143	0.36364	0.40692667

Pm166 X Pm167	0.57143	0.68293	0.64	0.63145333
Pm166 X Pm168	0.53846	0.63415	0.59259	0.5884
Pm166 X Pm169	0.51852	0.6	0.36364	0.49405333
Pm166 X Pm170	0.46154	0.59459	0.6087	0.55494333
Pm167 X Pm168	0.5	0.71429	0.5	0.57143
Pm167 X Pm169	0.48	0.63415	0.42105	0.51173333
Pm167 X Pm170	0.41667	0.57895	0.4	0.46520667
Pm168 X Pm169	0.43478	0.82927	0.47619	0.58008
Pm168 X Pm170	0.27273	0.57895	0.36364	0.40510667
Pm169 X Pm170	0.6087	0.48649	0.23529	0.44349333

Nest - 116

Nestlings - Pm229-232

Dyad	PER	33.15	33.6	MEAN
Pm229 X Pm230	0.74074	0.64706	0.66667	0.68482333
Pm229 X Pm231	0.41667	0.68421	0.60606	0.56898
Pm229 X Pm232	0.53846	0.5641	0.42857	0.51037667
Pm230 X Pm231	0.59259	0.47059	0.60606	0.55641333
Pm230 X Pm232	0.55172	0.51429	0.64286	0.56962333
Pm231 X Pm232	0.76923	0.76923	0.64	0.72615333

Nest - 82

Nestlings - Pm202-205

Pm202 X Pm203	0.71429	0.64286	0.41667	0.59127333
Pm202 X Pm204	0.66667	0.71429	0.73333	0.70476333
Pm202 X Pm205	0.61538	0.58333	0.56	0.58623667
Pm203 X Pm204	0.66667	0.8	0.6	0.68889
Pm203 X Pm205	0.61538	0.69231	0.48	0.59589667
Pm204 X Pm205	0.71429	0.76923	0.83871	0.77407667

Nest - 73

Nestlings - Pm160-164

Pm160 X Pm162	0.4	0.66667	0.63636	0.56767667
Pm160 X Pm163	0.6087	0.66667	0.7	0.65845667
Pm160 X Pm164	0.23077	0.55556	0.6087	0.46501
Pm162 X Pm163	0.7619	0.72727	0.58333	0.69083333
Pm162 X Pm164	0.41667	0.61538	0.74074	0.59093
Pm163 X Pm164	0.44444	0.55556	0.56	0.52

Nest - 95

Nestlings - Pm183,184,187

Pm183 X Pm184	0.6	0.4375	0.48276	0.50675333
Pm183 X Pm187	0.64286	0.57143	0.68966	0.63465
Pm184 X Pm187	0.69231	0.32258	0.57143	0.52877333