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Released sub-adult kaki feeding at plate.

(photo by Department of Conservation Twizel Area Office)
ABSTRACT

In order to maximise individual fitness, animals would be expected to pair with a mate of the highest possible quality. One measure of mate quality is relatedness, or genetic similarity. Choosing an unrelated, genetically dissimilar mate enriches the genetic resources of the offspring and reduces the chances that detrimental recessive alleles will be brought into homozygosity, causing inbreeding depression. In order to avoid mating with close kin, it is necessary for animals to be able to recognise kin. The mechanisms suggested for kin recognition include learning the characteristics of kin by association with them, or learning a kin template, which other individuals are judged against.

In small populations of endangered species, animals may be left with little choice of mates and as a result may be forced to breed with close relatives. One of the threats faced by small populations of endangered New Zealand birds, such as the kaki (*Himantopus novaezelandiae*), is that inbreeding will be detrimental to the population, reducing reproductive success or survival, or the ability of the population to adapt to environmental challenges.

Kaki are a critically endangered endemic wader reduced to one population in the Mackenzie Basin. The population has been actively managed for the past 20 years, using a variety of management techniques. Since 1993 multiple clutching has been combined with captive rearing and pulse releases of kaki and dark hybrids. The practise of multiple clutching means that not all siblings from a season are raised together, a situation that does not occur naturally as kaki usually only lay a second clutch if the first fails. In addition, some clutches are intermingled in captivity, so that some kaki are raised with unrelated birds. If kaki learn to recognise close siblings by association with them, the practise of multiple clutching presents kaki with siblings that they will assume to be unrelated and clutch intermingling presents kaki with unrelated birds they will assume are related. Management therefore has the potential to increase inbreeding, depending on the kin recognition mechanisms used by kaki.

By comparing the inbreeding levels in pairs involving released birds and wild-hatched birds, this study shows that management does not currently appear to be causing a high level of inbreeding. The tendency (although not statistically significant; $P= 0.1597$) for released birds to associate preferentially with siblings with whom they have been raised indicates that kin are probably learned primarily by association. Further work on kin recognition mechanisms in kaki is recommended to investigate the possibility that multiple clutching and clutch mixing will result in increased inbreeding and the relationship between breeding pairs should be monitored to determine whether increased inbreeding occurs. Possible inbreeding
can be minimised by aviary management and releasing siblings that have been raised separately into different sub-populations, although this may conflict with other management aims.

A short-term increase in inbreeding between full siblings may be justifiable to rapidly increase the population size. A larger population will then result in a reduced number of clutches from each pair being raised due to finite aviary space. Eventually the population should become large enough to eliminate the need for multiple clutching, and mating between full siblings will cease to be an issue. A short-term increase in sibling matings is therefore tolerable if it results in a long-term recovery of the species.
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Why choose a mate?

There are compelling reasons for believing animals choose a mate rather than mating indiscriminately (Halliday, 1983). Both sexual and natural selection would be expected to favour mechanisms that ensure animals choose mates according to their potential to enhance individual fitness (Halliday, 1983; Barnard & Aldhous, 1991). Mate choice is the behavioural mechanism central to Darwin’s (1871) theory of intersexual selection (Arnold, 1983; Barnard & Aldhous, 1991). Sexual selection can act through variation in the number of social mates obtained, variation in success at obtaining extra-pair fertilisations, or variation in mate quality (Webster et al., 1995). Quality may be measured by a variety of properties, including resources held, parenting ability, or genotype (Halliday, 1983).

Mate choice is defined as “any pattern of behaviour, shown by members of one sex, that leads to their being more likely to mate with certain members of the opposite sex than others” (Halliday, 1983). There are two possible strategies for choosing a mate, best of N and sequential sampling (Real, 1990) Best of N strategy involves a searching individual choosing the best possible mate out of a choice of N mates (Real, 1990). Sequential sampling involves a searching individual establishing a critical threshold and searching until a mate at or above the threshold is found (Real, 1990). Mate choice refers to observable behaviour, rather than referring to the animal’s internal state and covers a variety of phenomena, not all of which would be called choice in the human context (Halliday, 1983). In measuring mate choice, what we are actually recording is whether variations in behaviour are correlated with variations in mating success, rather than whether a true preference is involved (Halliday, 1983).

The various benefits to be gained by choosing one mate over another, such as higher fertility, better parental qualities, higher resource quality, good physical condition, or genetic dissimilarity (Halliday, 1983; Brown & Eklund, 1994) are all fitness enhancing. Pursuit of genetic dissimilarity in a mate enriches the genetic resources of offspring and may be achieved by the behavioural mechanisms of disassortive mating and inbreeding avoidance (Brown & Eklund, 1994).

Inbreeding

All populations may be considered inbred if traced back far enough, therefore, inbreeding is a relative concept (Ralls et al., 1986). Here I am concerned with inbreeding between close relatives, i.e. between siblings or between parents and offspring. Matings between close relatives are widely believed to cause inbreeding depression, a reduction in
fitness of inbred offspring, because they bring about increased homozygosity (Ralls et al., 1986; Part, 1996 & references therein). Increased homozygosity may be expected to cause inbreeding depression for three reasons (Ralls et al., 1986). First, the chances of a detrimental recessive allele being expressed are increased (Ralls et al., 1986). Second, a heterozygote may be fitter than either homozygote (Ralls et al., 1986), although not in every case. Third, decreased genetic variability may reduce the chance that an individual's offspring will survive a stochastic environmental event (Ralls et al., 1986; Keller et al., 1994; Dhont, 1996; Jamieson & Ryan, 2000).

Matings between close relatives are more likely to bring deleterious recessives into homozygosity than matings between unrelated individuals, because, although recessive alleles are usually rare in a population, some are likely to be present in recent common ancestors of closely related individuals (Part, 1996 and references therein). The phenotypic expression of the recessive alleles results in fitness depression (Ralls et al., 1986; Part, 1996), with the number of homozygous recessive alleles determining the level of fitness depression (Part, 1996).

Costs and benefits of inbreeding

Costs of inbreeding found in bird species include decreased reproductive fitness, (e.g. Japanese quail Coturnix coturnix japonica (Sittmann et al., 1966)), decreased hatching rate (e.g. Cooper's hawk Accipiter cooperii (Rosenfield & Bielefeldt, 1992)), decreased adult survival (e.g. song sparrows Melospiza melodia (Keller et al., 1994)) and higher hatching failure (e.g. takahe Porphyrio hochstetteri; Jamieson & Ryan, 2000). Costs found in other taxa include sperm deformities (e.g. lions Panthera leo (Packer & Pusey, 1993)) and decreased courtship frequency (e.g. amphibians and lizards Waldman & McKinnon, 1993). Studies on domestic animals have shown harmful effects on development, survival and growth rate (Templeton, 1986).

Costs of inbreeding may sometimes only be manifested in times of environmental stress (Keller et al., 1994; Dhont, 1996; Jamieson & Ryan, 2000). Therefore, care must be taken in drawing conclusions as to the success of recovery programs for endangered species, as inbred populations may be unable to react to a new environmental stress e.g. a new competitor or new parasite (Dhont, 1996). The interaction between environment and inbreeding depression is illustrated by lower hatching rates in takahe translocated to islands with different environmental conditions to the place of origin (Jamieson & Ryan, 2000) and lower survival rate in inbred song sparrows during a population crash caused by severe winter weather (Keller et al., 1994). However, the extent to which inbreeding threatens the continued existence of a species has been widely debated (Wallis, 1994). While there is ample evidence
that inbreeding increases mortality of early life stages there is little evidence that inbreeding increases mortality at a population level, or causes populations to go extinct (Dhont, 1996).

More recently, it has been recognised that accumulation of deleterious recessive alleles and consequent fitness costs of inbreeding are related to population size and history (Wallis 1994; Part, 1996; Pusey & Wolf, 1996), and the intensity of selection against the deleterious alleles (Dhont, 1996; Part, 1996). Small populations that have been subjected to a long history of inbreeding may have had time for deleterious recessive alleles to be unmasked and purged (Pusey & Wolf, 1996). Purging in small populations results in no, or small, fitness costs of close inbreeding when compared to larger, outbred populations, provided selection pressures and environmental conditions are the same as in the past (Dhont, 1996; Part, 1996). Long exposure to high levels of homozygosity may even confer benefits (Templeton, 1986; Butler & Merton, 1992), including maintaining locally adapted (Butler & Merton, 1992) or co-adapted gene complexes (Templeton, 1986), which give specific positive adaptations to a local situation (Butler & Merton, 1992).

Mayr (1963) suggested that most of the genes in a species are co-adapted and outbreeding reduces fitness by breaking up co-adapted gene complexes, but this idea is controversial (Templeton, 1986). The phrase ‘outbreeding depression’ is used to refer to fitness reductions caused by outbreeding, regardless of their cause, avoiding the controversy of associating co-adaptation with outbreeding depression (Templeton, 1986). The least controversial cause of outbreeding depression is local adaptation, in which different geographical populations of a species adapt to their local environments, particularly if dispersal is limited and the population is small for a long period of time (Templeton, 1986).

Outbreeding depression at the species level (hybrid breakdown) is common in the F2 generation (Harrison, 1993). Outbreeding depression can be intrinsic (e.g. a result of chromosome or genic incompatibility) or extrinsic (e.g. lack of environmental fit or behavioural incompatibilities of intermediates; Wallis, 1999). Therefore hybridisation presents two threats to endangered species, loss of integrity of the introgressed form and potential long-term loss of fitness as a result of hybridity (Wallis, 1999). However, reduced hybrid fitness is often restricted to one of the sexes (Wallis, 1999). Haldane’s Rule (1935) states that whenever one sex is absent, rare or sterile, it is the heterogametic sex (ZW female in birds).

If inbreeding and outbreeding both have negative fitness consequences, animals should choose mates of intermediate relatedness (Bateson, 1978; 1983; Barnard & Aldhous, 1991). Bateson (1978; 1983) suggested that animals learn the visual, auditory or olfactory characteristics of their immediate kin and choose a mate that is slightly different to those familiar from early life, thus achieving an optimal balance between inbreeding and
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outbreeding. Male Japanese quail (Bateson, 1978) and zebra finch (Taeniopygia guttata (Burley et al., 1990)) both display the predicted preference ranking for slightly unfamiliar over familiar or grossly unfamiliar.

Whereas in some situations inbreeding may be beneficial, as a general rule, inbreeding has fitness costs, therefore, animals should be expected to avoid inbreeding. However, avoiding inbreeding also entails costs, such as increased mortality risk when dispersing over unfamiliar territory and reduced reproductive success, probably due to a greater likelihood of poorer nest site choice in unfamiliar territory (Part, 1996). Therefore, animals should be expected to actively avoid inbreeding only if the costs of tolerance outweigh the costs of avoidance (Part, 1996).

Mechanisms of inbreeding avoidance and kin recognition

Inbreeding avoidance may be achieved by dispersal or by actively avoiding mating with kin (Ralls et al., 1986). Dispersal may involve one or both sexes moving away from the area in which they were born before breeding (natal dispersal) or adults moving between breeding seasons (breeding dispersal) (Ralls et al., 1986). Delayed return to breeding grounds, a behaviour exhibited by collared flycatchers (Ficedula albicollis), may be a variation of this mechanism, dispersing individuals in time rather than space (Part, 1996). Kin avoidance involves recognition of kin by various mechanisms (described below) and subsequent avoidance of kin in choosing a mate.

Avoidance of choosing kin as a mate is not the same as the neural process of recognition (Byers & Bekoff, 1986). Barnard & Aldhous (1991) suggest the use of the term kin discrimination for differential behavioural responses to kin resulting from kin recognition. Kin recognition is commonly differentiated into three components (Beecher, 1982; Gamboa & Pfennig, 1986; Waldman, 1988; Reeve, 1989; Gamboa et al., 1991), of which the first two involve actual recognition and the third involves discriminatory action. The expression component involves the nature and acquisition of recognition cues and the perception component involves the development of a recognition template, the sensory processing of recognition cues and the matching of the cues and template (Gamboa et al., 1991). The action component involves the discriminatory action taken following assessment of the similarity between the cues and template (Gamboa et al., 1991). Discrimination is a result of recognition, but absence of discrimination does not necessarily imply an absence of recognition. Recognition cues, actions and templates are often not fixed and may change during ontogeny or in different contexts (Gamboa et al., 1991). Uncoupling the components of kin recognition
allows for assessment of changes in each over time or in different contexts and may be achieved by methods outlined in Gamboa et al. (1991).

The various mechanisms suggested for kin recognition reflect the sensory capabilities of the species employing them, from pheromones in the lower invertebrates to sophisticated combinations of vision, sound and smell in the primates (Fletcher & Michener, 1987). Fletcher & Michener (1987) present three mechanisms of kin recognition; spatial distribution, allelic recognition, and phenotype matching, while Halpin (1991) adds a fourth, recognition by association. Kin recognition by spatial distribution may be used where there is a high probability that animals within a given location will be related to one another, but as it involves preferential treatment towards all others found within a given locality, it is not true kin recognition (Halpin, 1991). Allelic recognition involves recognition of phenotypic similarities correlating with kinship, which bias behavioural response in favour of individuals carrying similar alleles (Barnard & Aldhous, 1991). Allelic recognition differs from phenotype matching in that recognition cues will always have a genetic origin (Halpin, 1991). While most cues used for recognition by phenotype matching will have genetic origin, in some cases they may be environmentally acquired (Halpin, 1991). Phenotype matching is particularly useful for species that are likely to encounter unfamiliar individuals that may or may not be kin and involves discrimination based on comparison with a known kin phenotype (Halpin, 1991). Phenotype matching can be by comparison with a template based on nest-mates or self (self-referent matching (Todrank & Heth, 2001)). Recognition by association involves learning the characteristics of siblings during exclusive association with them as nest-mates (Halpin, 1991). Recognition by association is likely to occur in altricial hatchlings remaining together in the nest (e.g. Japanese quail (Bateson 1978)) but may also occur in precocial chicks, which remain in family groups after hatching (e.g. black stilts Himantopus novaezelandiae and banded dotterels Charadrius bicinctus).

It has commonly been assumed that animals use only one mechanism of recognition (Todrank & Heth 2001, but see Holmes & Sherman, 1982 and Ralls et al., 1986) and cross-fostering techniques were developed to distinguish between the importance of each method. However, cross-fostering tests are often biased towards familiarity, as animals tend to treat familiar and unfamiliar individuals differently regardless of whether they are relatives (Todrank & Heth, 2001). This problem can be avoided by raising test subjects with both kin and non-kin (Todrank & Heth, 2001). Non-random spacing (Bateson, 1982), non-random distribution of amicable acts (Kareem & Barnard, 1982), parental care (Beecher et al., 1981), odour preferences (Gilder & Slater, 1978), non-random nest admittance (Greenberg, 1979) and non-
random distribution of agonistic behaviour (Kareem & Barnard, 1982) have all been used to infer kin recognition.

Behavioural observations are the most commonly employed method of studying kin recognition, but are frequently prone to observer bias (Gamboa et al., 1991). While observer bias can be minimised by using observers blind to the relatedness of subjects, and where relevant, their pre-test treatment, bias may also enter behavioural studies through non-independence of data. Two common causes of non-independent data are multiple observations of individuals and treating related individuals as independent (Gamboa et al., 1991). Data from nest-mates is not independent as families may share behavioural responses due to the sharing of alleles and therefore the phenotypic characters for which they code (Barnard & Aldhous, 1991). As well as kin recognition, inbreeding levels and inbreeding avoidance have been studied using behavioural methods, although genetic methods have also been employed.

**Quantifying inbreeding and inbreeding avoidance**

Inbreeding avoidance has been studied using three major methods (Part, 1996). First, observing individual behaviour in the presence and absence of close kin (e.g. Burley et al., 1990; TenCate & Bateson, 1988). Second, contrasting costs of avoidance with costs of tolerating close inbreeding (e.g. Part, 1996, Waser et al., 1986). Third, comparing observed and random frequencies of close inbreeding (Part, 1996). While methods of investigating inbreeding levels and whether or not animals avoid inbreeding have concentrated on direct observations of behaviour in the past (Part, 1996), the development DNA isolating techniques provides an indirect but more accurate way to quantify inbreeding.

Part (1996) suggested behavioural observations are the strongest evidence for inbreeding avoidance as they directly measure an animal's choice of mate, and can involve experimental manipulation. However, Dobson & Jones (1985) rejected behavioural observations on the grounds that they may reflect past selection pressures. This may well be the case for New Zealand birds, which have reduced population sizes and distributions since human colonisation, possibly leading to inbreeding levels higher than those found historically. As behavioural studies usually take place in laboratory environments with only limited choice between individuals, results may not be directly applicable to the wild situation where birds have a far greater choice of potential mates. However, it is difficult to obtain reliable data from behavioural observation studies in the wild, as field studies generally do not detect extra-pair copulations and egg dumping, due to logistical constraints on the amount of time spent
on observation, and the furtive nature of extra-pair copulations. Consequently, observed levels of inbreeding may be different to actual levels.

Contrasting costs of inbreeding avoidance or tolerance indirectly tests whether inbreeding avoidance should be expected to occur (Part, 1996). This method is unable to prove whether or not inbreeding avoidance does actually occur. Theoretically, animals should only be expected to avoid inbreeding if the costs of tolerance are higher than the costs of avoidance (Part, 1996). When comparing costs of inbreeding avoidance and tolerance, estimates must be made of the costs involved, as it is difficult to measure them. Part (1996) compared reproductive success of philopatric and dispersing collared flycatchers and found that avoiding close inbreeding by dispersal has a substantially greater cost than tolerating inbreeding.

Comparisons of observed and expected levels of inbreeding are unreliable as they may give different results depending on the null model used (Ralls et al., 1986; Part, 1996). The null model should be independent of inbreeding avoidance behaviours, and unless independence is achieved, comparisons are invalid (Ralls et al., 1986; Part, 1996). For example, Part (1996) found that collared flycatchers avoid inbreeding when using a null model that took into account different arrival times at the breeding ground, but no inbreeding avoidance was found when using a completely random null model. The first null model assumed that arrival time is not an inbreeding avoidance mechanism and incorporates arrival time into the model. The second null model assumed that arrival was timed to avoid inbreeding and so did not incorporate arrival time into the model. Unfortunately, it was not possible to determine whether inbreeding avoidance or other factors cause different arrival times, so whether or not arrival time should be incorporated into the null model is debateable (Part, 1996). The same principle applies to other behaviours, such as dispersal, which may or may not be mechanisms of inbreeding avoidance. Whatever the null model used, comparisons of observed and expected inbreeding are liable to be inaccurate for the same reasons behavioural observation studies yield unreliable data; extra-pair copulations and egg dumping make observed parentage unreliable.

Genetic studies require all birds within the study population to be captured so that tissue samples can be taken for DNA analysis. The incorporation of genetic work into observational studies greatly strengthens the data, by allowing the inbreeding coefficient (the degree of co-occurrence of pairs of alleles) to be measured. The value of genetic work to either confirm or contradict behavioural observation studies is demonstrated by the substantially greater inbreeding levels in blue duck found by genetic analysis (>50%; Triggs et al., 1992) than behavioural study (>15%; Williams, 1991). The advent of genetic techniques
has potential to make behavioural observation studies on inbreeding redundant, as DNA analysis determines parentage more reliably than behavioural observation. However, the large time commitment required for, and difficulty in, capturing birds, as well as the potential to stress or damage them and the expense of tissue analysis frequently prohibits the use of this technique. In addition, currently available molecular genetic techniques differ considerably with respect to versatility, cost, sampling requirements and development time for each new species (Chambers & MacAvoy, 1999).

Inbreeding in New Zealand birds

Theory suggests that, due to unmasking and purging of severely deleterious recessive alleles (Dhont, 1996; Part, 1996; Pusey & Wolf, 1996), many of New Zealand's endangered birds should not experience severe inbreeding depression, given their long history of small populations. The fate of the Chatham Island black robin (*Petroica traversi*) illustrates that while inbreeding may or may not have been historically 'normal', extreme levels of inbreeding do not automatically cause detrimental effects or extinction of species in the short term (Ardern & Lambert, 1997), although the medium-term effects of extreme inbreeding remain to be seen. The black robin population consisted of less than 30 individuals during an approximately 90 year confinement to Little Mangere Island, before going through a bottleneck of only one breeding female and two males following translocation of the population to Mangere Island (Butler & Merton, 1992). The population has since recovered to over 200 individuals and following further translocation another population has been established on South East Island (Butler & Merton, 1992). Black robins show no immediate ill effects from their extreme inbreeding, having high egg fertility (90%), hatchability (80%) and survival to 1 year (70%) (Butler & Merton, 1992). However, given the low genetic variability in the population, the robin's ability to adapt to possible future environmental changes has been severely reduced and the robin's long-term future prospects are uncertain. Low genetic variation appears to be a feature of some of the Chatham Island avifauna (Lambert *et al*., 2000), as would be expected of small populations from which deleterious recessive alleles have been purged (Part, 1996; Pusey & Wolf, 1996). Therefore, the lack of negative effects of inbreeding in black robins should not be directly extrapolated to mainland populations, which although they may now be small, were historically much larger.

Where inbreeding levels in mainland New Zealand birds have been studied, they have been found to be high. Studies on four mainland species (saddleback *Philesturnus carunculatus* by Craig & Jenkins, unpublished; tui *Prosthemadera novaeelandiae* by Stewart, 1980; blue duck *Hymenolaimus malacorhynchos* by Williams, 1991 and Triggs *et al*., 1992; pukeko *Porphyrio porphyrio*
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by Craig & Jamieson, 1988) found inbreeding levels ranging from >8% of pairs to >70% of pairs. In all of these cases, population sizes are reduced and distributions have changed since human colonisation of New Zealand (Heather & Roberstson, 1996), potentially increasing inbreeding, although the pukeko has a naturally incestuous mating system (Craig & Jamieson, 1988). Dispersal patterns, which have the potential to reduce inbreeding, have probably also changed as a result of reduction and fragmentation of habitat. Therefore, inbreeding levels of today may in many, but not all cases, be a result of human-induced selection pressures.

Whether managers should maintain several isolated populations of endangered birds rather than artificially promoting geneflow between them, or conserve one large population, remains open to debate. The benefit of maintaining several isolated inbred populations is that complete isolation of individual populations preserves rare alleles in a homozygotic state (Lacy, 1987). Maintaining separation also allows specific local genetic adaptation to occur (Butler & Merton, 1992), preserving an 'evolutionary species' (lineage that shares a common evolutionary fate, after Templeton, 1986) rather than a snapshot of past genetic structure that may be inappropriate to present habitat (Craig, 1988). However, large populations are believed to be at lower risk of extinction than small populations (Frankel & Soule, 1981) so one large population may be 'safer' than several small populations, although the reverse may also be true, as the maintenance of several small populations spreads the risk of extinction. Of course the ideal is always to have several large populations.
Background

There are two species of stilt (*Himantopus* spp.) in New Zealand. They are thought to derive from two separate colonisation events by the ancestors of pied stilts (*H. himantopus leucocephalus*) from Australia (Wallis, 1999). Ancient colonisation by stilt ancestors (up to one million years ago) is thought to have led to the evolution of the endemic kākī (black stilt *H. novaeseelandiae*; Wallis, 1999). Historically, kākī were present throughout New Zealand (Holdaway, 1995; Pierce, 1984a), but they are now confined to the lakes, rivers and wetlands of the Mackenzie Basin (Figure 1). The contraction of their range and reduction in their numbers is attributed primarily to predation by introduced mammalian predators (e.g. feral cats *Felis catus*, mustelids *Mustela* spp., rats *Rattus* spp.), exacerbated by habitat loss and modification (Pierce, 1982; 1986).

Kākī are further threatened by introgression from the pied stilt (Wallis, 1999), following the reinvansion of pied stilts approximately 150 years ago (Wallis, 1999; MacAvoy & Chambers, 1999). While the introduction of pied stilts was undoubtedly natural, their subsequent rapid range expansion at the expense of kākī is probably largely due to habitat modification through land clearance and river management (Pierce, 1982; 1986), to which pied stilt have a greater tolerance than kākī (Robertson *et al.*, 1983). Kākī and pied stilts can hybridise, resulting in offspring of intermediate plumage (Pierce, 1984b). The likelihood of hybrid matings is increased by the low density of the kākī population limiting contact with potential mates and a sex imbalance (2.4:1 male bias (Maloney & Murray, in press)). Pierce (1984b) sub-divided kākī, hybrids and pied stilts into ten 'nodes', based on
adult plumage. Node A represents the 'pure' pied stilt genotype and node J the 'pure' kākā genotype, while nodes B to I represent hybrids with increasing proportions of kaki genes (Pierce, 1984b).

In addition to threats from predators, habitat loss and hybridisation, kākā's small population size makes it vulnerable to stochastic threats. Stochastic threats can be divided into four broad classes (Gilpin & Soulé, 1986; Shaffer, 1987). First, demographic uncertainty resulting from random events in the survival and reproduction of individuals; second, environmental uncertainty due to unpredictable changes in weather, food supply etc; third, natural catastrophes such as floods, fires etc and fourth, genetic uncertainty due to inbreeding, which alters the survival and reproductive probabilities of individuals (Gilpin & Soulé, 1986; Shaffer, 1987). These stochastic threats also interact; for example, inbred individuals may be more prone to extinction caused by environmental stochasticity (Gilpin & Soulé, 1986; Shaffer, 1987; Keller et al., 1994; Dhont, 1996; Jamieson & Ryan, 2000).

In the past, research on kaki genetics (Chambers & MacAvoy, 1999; Wallis, 1999) has concentrated on whether kaki and pied stilts are separate species. However, concern over the effect of inbreeding on small populations of New Zealand species has increased in recent years because of evidence that inbred animals show reduced fitness (Wallis, 1994). While there is no need to undertake further genetic analysis to examine questions about kaki's specific status (Maloney, 1999; Maloney & Murray, in press), inbreeding in kaki has not yet been addressed.

Breeding biology

The kaki population currently exists as a single breeding population in the Mackenzie Basin (Reed et al., 1993) although it is divided into geographically separate sub-populations (Sanders, 1996; this study). While most kaki spend their lives within one sub-population range, infrequent movements between sub-populations occur (Sanders, 1996).

Breeding pairs are typically socially monogamous, although trios of one male and two females, and female-female pairs have occurred. Kaki have high mate fidelity (Maloney & Murray, in press), so kaki already paired from the previous season are unavailable for forming new pairs. The population's strong male bias (Maloney & Murray, 2001) results in a large number of unpaired 'floater' males. Floater males are known to seek extra-pair copulations in other species (e.g. tree swallows Tachycineta bicolor; Barber & Robertson, 1999), but it is not known whether kākā engage in extra-pair copulations. However, as most offspring are the
expected node intermediate between the two parent nodes, extra-pair copulations are probably very rare, if they occur at all.

Kakī usually lay one clutch of four eggs per season but lay another clutch if the first fails (Pierce, 1982). Kakī chicks are precocial but in the wild typically remain in the family group for about nine months until they are sub-adults (Pierce, 1982), potentially allowing them to learn to recognise their siblings by association with them. Kin may also be learned by spatial distribution, as kaki breeding pairs defend territories. Because of low plumage variability, kin recognition by phenotype matching is unlikely to occur using visual cues. However, recognition may be by other phenotypic traits, such as olfactory or audio cues. Kakī fostered to pied and hybrid parents imprint on them and follow them in migration from the Mackenzie Basin (Reed et al., 1993), suggesting that if phenotypic or allelic recognition do occur, they are weaker in kaki than the other methods of kin recognition. Recognition of kin by any, or a combination, of these methods potentially allows kaki to avoid inbreeding. While inbreeding and inbreeding avoidance have not been studied in kaki, fitness costs associated with outbreeding have been proven.

With one known exception, fledgling and two-year-old survival rates are lower in matings involving nodes F, G and H as one of the parents than they are in JxJ or JxI matings (Wallis, 1999). There is no evidence that reduced hybrid fitness is caused by management, but, as predicted by Haldane's Rule (1935), female hybrids show reduced offspring success, while there is no discernible effect in males (Wallis, 1999). Kaki show strong assortive mating (70% of node J pair with another node J despite their comprising only 3% of the population (Pierce, 1982; 1984)) suggesting avoidance of outbreeding. However, positive assortment could be an effect of the absence of the migratory pied stilts at the time mass form pair bonds (between July and November), rather than mate choice per se (Wallis, 1999). Strong outbreeding avoidance could indicate that outbreeding has greater fitness costs to kaki than inbreeding, although kaki may potentially seek mates of intermediate relatedness. Wallis (1999) considers inbreeding less important to the probability of kaki's long-term survival than factors such as habitat loss and predation. However, inbreeding should be avoided or minimised where possible in captive-reared birds (Wallis, 1999).

**Kakī Management**

Before management commenced, the kaki population had declined to around 23 adults (Maloney & Murray, in press). In 1981, the New Zealand Wildlife Service (now the Department of Conservation) began an intensive management program to increase fledging success and recruitment of juveniles (Pierce, 1996; Maloney & Murray, in press). Management
actions since then have included protection of eggs by artificial incubation, multiple clutching, cross-fostering to pied and hybrid parents, predator control, captive breeding, releases of captive-reared kākī and dark hybrids, habitat enhancement and protection, advocacy on disturbance and control of mixed pairs (Pierce, 1996; Maloney & Murray, in press). Various combinations of these management actions resulted in higher fledging and breeding (nesting plus fledging) success (from 6.1% fledging and 0.9% breeding success in unmanaged nests in the wild between 1977 and 1979 to 17.0% fledging and 16.5% breeding success for artificially incubated hatching eggs placed in the wild between 1992 and 1999 (Maloney & Murray, in press)). However, recruitment rates were much lower (4% of chicks hatched in the wild reached two years of age between 1992 and 1999 (Maloney & Murray, in press)).

Since 1981, there have been four major changes in management emphasis (Maloney & Murray, in press). Cross fostering ceased after 1987, when the emphasis changed to using high quality parents (node J) to produce offspring with a greater probability of being recruited to the breeding population (Reed et al., 1993; Maloney & Murray, in press). From 1993 to 1998 multiple clutches were obtained from wild and captive breeding pairs and incubated in captivity, after which half were then reared in captivity for release in small groups into the wild and half were returned to wild pairs as hatching eggs (Reed, 1998; Maloney & Murray, in press). From 1998 multiple clutches have been obtained from wild and captive pairs and all eggs have been hatched and reared in captivity for release into the wild (Maloney & Murray, in press). Initially, kākī were released as sub-adults, but since 1999 additional releases of juveniles have occurred, allowing a greater number of kākī to be reared each year. Following release, supplementary food (ox heart, insectivore mix and mealworms) is provided for about four to eight weeks, which along with the addition of iodine to the diet pre-release, is credited with the improved survival rates since 1998 (Maloney & Murray, in press). The first phase of the current Kākī Recovery Plan is to increase the number of kākī in the wild in the Mackenzie Basin, while the future vision of the plan is for kākī to be found in thriving associations in habitats throughout New Zealand (Maloney & Murray, in press).

Potential problem caused by kākī management

During artificial incubation and rearing in captivity clutches are occasionally separated and/or intermingled. Because of multiple clutching and clutch separation or intermingling at the aviary, some kākī are raised with their full siblings (known siblings), some kākī are raised separately to full siblings (unknown siblings) and some are raised with unrelated cage-mates (cage-mates).
Assuming kakī usually avoid mating with close kin, clutch manipulation potentially affects the formation of breeding pairs involving captive-raised kakī in two ways. First, if kakī are raised apart from siblings, they may fail to recognise them as kin, and therefore, fail to avoid mating with them, resulting in an increase in inbreeding among released birds. Second, if kakī are raised with unrelated cage-mates they may perceive them to be siblings and avoid selecting them as mates. This may result in a reduction in the number of unrelated birds available as mates, and subsequent increase in the probability of inbreeding.
Aims

This study has three broad aims. First, to quantify inbreeding levels in released kaki. Second, to compare inbreeding levels in released kaki to inbreeding levels in wild-hatched kaki and expected levels of inbreeding given random mating. Third, to identify which, if any, kin recognition mechanisms are employed by kaki.

Specific objectives

This study will:
1) assess the inbreeding level in paired released kaki,
2) assess the inbreeding level in paired wild-hatched kaki,
3) compare the inbreeding levels in paired released and wild-hatched kaki,
4) compare observed and expected frequencies of close inbreeding among released kaki using a random mating null model,
5) test for kin recognition by association by comparing observed and random frequencies of association between released kaki, known siblings and cage-mates,
6) test for kin recognition by phenotype matching by comparing observed and random frequencies of association between released kaki and unknown siblings,
7) establish whether association with kin changes over time.
STUDY DESIGN AND METHODS

Department of Conservation (DOC) staff at the Twizel Area Office have kept comprehensive databases of aviary, breeding and sighting records since 1982. These databases provide all the information necessary to carry out this study.

Most of the kakī population, and all released kakī or dark hybrids, are individually banded and identifiable. Currently only two of 60 adult birds are unbanded and band loss is minimal (R. Maloney, pers. comm.). Unbanded kakī may be hatched and raised in the wild without being banded due to nests being undetected or on property where access is denied, or have been banded but have subsequently lost their bands. Numbers of unbanded birds are often higher in previous years. I assume all bands are read correctly and as less than one percent of records in the sightings database are of birds with unidentified bands, I considered misidentification minimal. I assume no extra-pair copulation or egg dumping and that observed parentage is reliable. The assumption of no extra-pair copulation is reasonable because of the consistent throwing of hybrid offspring with nodes intermediate between parent nodes. The absence of clutches larger than the maximum four eggs possible for a single kakī pair suggests that egg dumping does not occur. I assume minimal movement between sub-populations, based on Sanders (1996) study.

All data was entered into Microsoft Office Excel spreadsheets and an MS DOS program was constructed to perform randomisation tests.

Mate choice

Assess observed inbreeding level in paired released kakī

I compiled a list of all 39 pairs involving one or two released kakī or dark hybrids (hereafter released birds) that were recorded in the kakī database between 1994 and 2000. Prior to 1993 no released birds were hand reared and between 1987 and 1993 there was no multiple clutching, so there was no potential for management to increase inbreeding. In 1993 multiple clutches were hand-reared, but the released birds from 1993 were not old enough to breed till 1994. Pairs involving one released and one wild-hatched kakī or dark hybrid (hereafter wild-hatched birds) were included in estimates of inbreeding levels for both released and wild-hatched birds.

Where possible, I used the kakī family tree and aviary records to establish whether pairs involved full siblings or not, and whether the pair had been cage-mates during captive-rearing. As only full sibling matings are considered, this study is likely to underestimate inbreeding.
Pairs were only counted once; pairs staying together in subsequent years were not recounted. There was a female-female pair, involving one released bird that I excluded. Where parentage of the released birds’s mate was unknown, I compared their node and the sub-population they came from (see determining sub-populations below) to determine whether it was possible for them to be siblings. Birds of different nodes could not be full siblings. Birds of the same node could not be siblings if they came from different sub-populations, but if they came from the same sub-population their relationship was recorded as unknown. In two cases it was not possible to determine whether the pair involved siblings, as both members of the pair were the same node and the released birds had come from the same sub-population as its mate, which was banded as an adult and had unknown parentage. For the records where the relationship between the pair was known, I calculated the proportion of pairs involving sibling matings each year and the total across all years.

Assess observed inbreeding level in paired wild-hatched kākā

I compiled a list of all 98 pairs involving one or two wild-hatched birds reared by their own or foster parents after incubation in captivity, with one exception that was not incubated in captivity) that were recorded in the black stilt database between 1985 and 1999. I assume wild-hatched birds represent the natural state, as they are raised with only full siblings. Where possible, I used the kākā family tree to establish whether pairs involved full siblings or not.

Pairs were only counted once and I excluded the same female-female pair as in the released bird category, which also involved a wild-hatched bird. Where parentage of the wild-hatched bird’s mate was unknown, I compared their node and sub-population. In 24 cases it was not possible to establish whether the pair were siblings or not as both birds were the same node and either one of the pair had been banded as an adult and its parentage was unknown, or the parents of both were unbanded and from the same sub-population. For the records where the relationship between the pairs was known, I calculated the proportion of pairs involving sibling matings each year and the total across all years.

Determining sub-populations

Observations of black and dark hybrid stilts are categorised in the black stilt database into 16 areas. An analysis to group these areas into sub-populations was necessary for this study to determine whether birds of the same node and unknown parentage could be siblings and to determine which kākā or dark hybrids were available as potential mates for released birds.

Here, I define the geographic ranges of three sub-populations of kākā and dark hybrids, based on a cluster analysis of the 16 areas defined by DOC. I used cluster analysis to group the 16 areas recognised by DOC on the basis of recorded sightings of colour banded
kakī and dark hybrids. At the time of this analysis (October 2001), 16,828 sightings of 587 kakī were recorded between September 1982 and March 2001 in the kakī database at DOC’s Twizel Area Office. I clustered Pearson’s correlation coefficients using Unweighted Paired Groups Method of Arithmetic Averages (UPGMA). Birds observed on less than ten occasions and areas irrelevant to this study (e.g. North Island) were excluded from the analysis.

*Compare observed inbreeding levels in paired released and wild-hatched kakī*

Inbreeding levels in paired released and wild-hatched birds could only be compared qualitatively. It was not possible to perform statistical analysis due to the large number of pairs of unknown relationship (two released bird pairs and 24 wild-hatched bird pairs). I calculated the maximum possible probability of sibling matings by assuming all pairs of unknown relationship were siblings, and likewise, I calculated the minimum possible probability by assuming all pairs of unknown relationship were not siblings.

*Compare observed and expected frequencies of inbreeding among released birds*

I compiled a list of all kakī and dark hybrids seen in each sub-population between 1 July and 30 November each year (during which time breeding pairs mostly form), and therefore, available as a potential mate for released birds, from 1994 to 2000. Birds already in a pair from the previous year were excluded as they do not break already existing pair bonds. Juveniles were excluded as they do not breed. Unidentified banded birds were excluded. Due to the difficulty in knowing how many unbanded birds are in an area, unbanded birds were included only if they later formed a breeding pair. Birds paired earlier in the same season were considered available, as all birds potentially intermingle during pair formation (c.f. Part, 1996 who did not include birds paired earlier in the season due to different arrival times at the breeding ground).

Statistical analysis was not possible due to the high proportion of birds with unknown sex and/or parentage. This was more of a problem for older wild-hatched birds than released birds.

*Kin recognition*

I compiled a list of groups of released birds observed up to 1 month after each release between 2000 and 2001. After the first month, observations are less frequent and kakī numbers decline because of mortality or dispersal (Figure 2). Earlier releases are not included due to higher mortality. The definition of what constitutes a group is subjective, but is assumed to be reasonably constant between observers. Groups are generally separated by fairly large distances, but where several groups are seen in the same area, they may be distinguished by movement together as a group or aggressive interaction between groups.
**Figure 2.** Kaplan-Meier survival curves showing the cumulative proportion of released birds surviving for 40 days post-release. (source: Department of Conservation Twizel Area Office)

**Test for kin recognition by association and/or phenotype matching**

I compared the observed relationships within groups of released birds with the probability of their occurrence by chance. I used randomisation tests to generate a probability of association with siblings, cage-mates and siblings who had also been cage-mates. Randomisation tests were run for 10000 replicates for each group size from each release. The null hypothesis of random association between siblings, cage-mates and sibling cage-mates was accepted or rejected at the 5% level.

**Establish whether association with kin changes over time**

I observed groupings of released juvenile and sub-adult birds over an 11km stretch of the Ahuriri River and adjacent wetlands between 18 April 2001 and 24 April 2001, three months after release of the juveniles and seven months after the release of the sub-adults. I compared the observed relationships within the groups of released birds with the probability (generated by a randomisation test) of their occurrence by chance. The randomisation test was run for 10000 replicates for each group size from each release. The null hypothesis of random association between siblings, cage-mates and sibling cage-mates was accepted or rejected at the 5% level.
RESULTS

**Inbreeding levels in released and wild-hatched birds**

I was unable to determine the relationship between two released pairs and 24 wild-hatched pairs as both birds of these pairs were the same node and from the same sub-population and one or both had unknown parentage (Table 1). I was able to determine the relationship of 37 pairs involving released birds and 74 pairs involving wild-hatched birds (Table 1).

Table 1. Numbers of released and wild-hatched bird pairs of known and unknown relationship. “No pairs” indicates no pairs involving released birds in that particular season.

<table>
<thead>
<tr>
<th>season</th>
<th>released birds pairs</th>
<th>wild-hatched birds pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>relationship known</td>
<td>relationship unknown</td>
</tr>
<tr>
<td>1985</td>
<td>no pairs</td>
<td>1</td>
</tr>
<tr>
<td>1986</td>
<td>no pairs</td>
<td>0</td>
</tr>
<tr>
<td>1987</td>
<td>no pairs</td>
<td>1</td>
</tr>
<tr>
<td>1988</td>
<td>no pairs</td>
<td>10</td>
</tr>
<tr>
<td>1989</td>
<td>no pairs</td>
<td>3</td>
</tr>
<tr>
<td>1990</td>
<td>no pairs</td>
<td>6</td>
</tr>
<tr>
<td>1991</td>
<td>no pairs</td>
<td>10</td>
</tr>
<tr>
<td>1992</td>
<td>no pairs</td>
<td>8</td>
</tr>
<tr>
<td>1993</td>
<td>no pairs</td>
<td>11</td>
</tr>
<tr>
<td>1994</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1995</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>1996</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>1997</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>1998</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>1999</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>2000</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>total</td>
<td>37</td>
<td>2</td>
</tr>
</tbody>
</table>

For pairs of known relationship the observed probabilities of released and wild-hatched bird pairs being between full siblings are summarised in Table 2. The total inbreeding level across all years was the same in wild-hatched birds and released birds, although sample sizes were not the same. There was one sibling mating involving a captive-raised, released bird and it’s wild-hatched sibling in 2000. There were no pairs involving unrelated cage-mates. There were two sibling matings involving wild-hatched birds. One involved the wild-hatched
bird mated with its released sibling previously mentioned. The other sibling mating in 1988 involved two wild-hatched birds raised together by their own parents.

Table 2. The observed probabilities of pairs involving one or two released and wild-hatched birds being between siblings (number of total pairs in brackets). “No pairs” indicates no pairs involving released birds in that particular season. “Relationship unknown” indicates there were pairs involving wild-hatched birds in that season, but their relationship was undetermined.

<table>
<thead>
<tr>
<th>season</th>
<th>released birds pairs</th>
<th>wild-hatched birds pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1985</td>
<td>no pairs</td>
<td>0.000 (1)</td>
</tr>
<tr>
<td>1986</td>
<td>no pairs</td>
<td>relationship unknown</td>
</tr>
<tr>
<td>1987</td>
<td>no pairs</td>
<td>0.000 (1)</td>
</tr>
<tr>
<td>1988</td>
<td>no pairs</td>
<td>0.100 (10)</td>
</tr>
<tr>
<td>1989</td>
<td>no pairs</td>
<td>0.000 (3)</td>
</tr>
<tr>
<td>1990</td>
<td>no pairs</td>
<td>0.000 (6)</td>
</tr>
<tr>
<td>1991</td>
<td>no pairs</td>
<td>0.000 (10)</td>
</tr>
<tr>
<td>1992</td>
<td>no pairs</td>
<td>0.000 (8)</td>
</tr>
<tr>
<td>1993</td>
<td>no pairs</td>
<td>0.000 (11)</td>
</tr>
<tr>
<td>1994</td>
<td>0.000 (1)</td>
<td>0.000 (6)</td>
</tr>
<tr>
<td>1995</td>
<td>0.000 (2)</td>
<td>0.000 (4)</td>
</tr>
<tr>
<td>1996</td>
<td>0.000 (5)</td>
<td>0.000 (2)</td>
</tr>
<tr>
<td>1997</td>
<td>0.000 (11)</td>
<td>0.000 (4)</td>
</tr>
<tr>
<td>1998</td>
<td>0.000 (5)</td>
<td>0.000 (3)</td>
</tr>
<tr>
<td>1999</td>
<td>0.000 (6)</td>
<td>0.000 (4)</td>
</tr>
<tr>
<td>2000</td>
<td>0.143 (7)</td>
<td>1.000 (1)</td>
</tr>
<tr>
<td>total</td>
<td>0.027 (37)</td>
<td>0.027 (74)</td>
</tr>
</tbody>
</table>

Assuming all pairs of unknown relationship are between siblings generates the maximum possible probabilities of released and wild-hatched pairs involving full siblings (Table 3). Likewise, assuming all pairs of unknown relationship are not siblings generates the minimum possible probabilities of pairs involving siblings (Table 3). The greater number of wild-hatched pairs of unknown relationship causes the difference between the maximum and minimum probability of inbreeding in wild-hatched pairs to be much greater than the difference between the maximum and minimum probability of inbreeding in released birds. In reality, the actual probabilities of pairings involving siblings lies somewhere between each of these extremes.
Table 3. The maximum and minimum possible probabilities of pairs involving one or two released or wild-hatched birds being inbred if pairs of unknown relationship are assumed to be siblings (maximum) and not siblings (minimum).

<table>
<thead>
<tr>
<th>released birds pairs</th>
<th>maximum</th>
<th>known</th>
<th>minimum</th>
<th>wild-hatched birds pairs</th>
<th>maximum</th>
<th>known</th>
<th>minimum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.077</td>
<td>0.027</td>
<td>0.026</td>
<td></td>
<td>0.265</td>
<td>0.027</td>
<td>0.020</td>
</tr>
</tbody>
</table>

Expected inbreeding level in released birds

Sub-population analysis

The results of the cluster analysis are presented as a dendrogram in Figure 3. I recognised three geographic sub-populations on the basis of three clusters of areas:

Sub-population A: (southern)

Sub-population B: (central)

Sub-population C: (northern)

Figure 3. Dendrogram showing the grouping of 11 areas into three geographic sub-populations, A (southern) B (central) and C (northern) using Unweighted Paired Groups Method of Arithmetic Averages (UPGMA). The dashed line shows the linkage distance at which sub-populations were distinguished.

It was not possible to calculate expected inbreeding levels as the sex of 20% of available birds was unknown and 22% of available birds had unknown parentage.

Kin recognition

Approximately 92% of released birds had been reared with siblings in captivity, approximately 38% had siblings that were raised separately and approximately 33% had been raised with cage-mates that were unrelated to them (Table 4).
Table 4. Numbers of released birds raised with siblings, separately to siblings and/or raised with unrelated cage-mates. These figures do not sum to 100% as some birds fell into two or three categories.

<table>
<thead>
<tr>
<th>release group</th>
<th>raised with siblings</th>
<th>raised separately to siblings</th>
<th>raised with unrelated cage-mates</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 Jan 2001</td>
<td>9 / 9</td>
<td>6 / 9</td>
<td>0 / 9</td>
</tr>
<tr>
<td>18 Jan 2001</td>
<td>19 / 20</td>
<td>8 / 20</td>
<td>10 / 20</td>
</tr>
<tr>
<td>12 Sep 2000</td>
<td>14 / 16</td>
<td>7 / 16</td>
<td>0 / 16</td>
</tr>
<tr>
<td>7 Feb 2000</td>
<td>5 / 6</td>
<td>0 / 6</td>
<td>3 / 6</td>
</tr>
<tr>
<td>17 Jan 2000</td>
<td>25 / 27</td>
<td>9 / 27</td>
<td>13 / 27</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>72 / 78</strong></td>
<td><strong>30 / 78</strong></td>
<td><strong>26 / 78</strong></td>
</tr>
</tbody>
</table>

Association between siblings, cage-mates and sibling cage-mates was not significantly different to random when averaged over five releases (Table 5). However, there was significant association between sibling cage-mates in two of the releases and between cage-mates in one of the releases (Table 5), suggesting that birds tend to preferentially associate with siblings or other birds with whom they have been raised.

Table 5. Randomisation test results for association between: 1) observed groups of siblings, cage-mates and sibling cage-mates one month post-release for five releases between 17 Jan 2000 and 24 Jan 2001; 2) average of all five releases; 3) observed groups three months after 18 Jan 2000 release and 4) observed groups seven months after 12 Sep 2000 release. (Significant values **bold**.)

<table>
<thead>
<tr>
<th>release group</th>
<th>one month post-release</th>
<th>three months post-release</th>
<th>seven months post-release</th>
<th>probability of association with sibling</th>
<th>cage-mate</th>
<th>sibling cage-mate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 Jan 2001</td>
<td>18 Jan 01</td>
<td>12 Sep 00</td>
<td>0.3505</td>
<td>0.4787</td>
<td>0.1647</td>
</tr>
<tr>
<td></td>
<td>18 Jan 2001</td>
<td>0.2671</td>
<td>0.6134</td>
<td>0.3017</td>
<td>0.7915</td>
<td>0.2016</td>
</tr>
<tr>
<td></td>
<td>12 Sep 2000</td>
<td>0.1808</td>
<td>0.6198</td>
<td>0.3427</td>
<td>0.4056</td>
<td>0.1597</td>
</tr>
<tr>
<td></td>
<td>7 Feb 2000</td>
<td>0.3017</td>
<td>0.4801</td>
<td>0.3427</td>
<td>0.4056</td>
<td>0.1597</td>
</tr>
<tr>
<td></td>
<td>17 Jan 2000</td>
<td>0.3427</td>
<td></td>
<td>0.3427</td>
<td>0.4056</td>
<td>0.1597</td>
</tr>
<tr>
<td>average of five releases</td>
<td></td>
<td></td>
<td></td>
<td>0.3427</td>
<td>0.4056</td>
<td>0.1597</td>
</tr>
<tr>
<td>three months post-release</td>
<td>18 Jan 01</td>
<td>0.4457</td>
<td>0.4204</td>
<td>0.4457</td>
<td>0.3280</td>
<td>0.2268</td>
</tr>
<tr>
<td>seven months post-release</td>
<td>12 Sep 00</td>
<td>0.4204</td>
<td>0.4107</td>
<td>0.4204</td>
<td>0.4107</td>
<td>0.1850</td>
</tr>
</tbody>
</table>
DISCUSSION

Captive-rearing and release management does not appear to be causing a high level of inbreeding between closely related released birds, as illustrated by the similarity of inbreeding levels in released and wild-hatched birds (Table 2). This reinforces confidence that management resulting in a population eventually comprising entirely captive-reared released birds will not be detrimental to the species. However, by including the sibling mating between the released bird and wild-hatched bird in the result for wild-hatched birds, I may have biased the result. This mating should possibly not have been included in the wild-hatched category, as it may have occurred because of release management. The birds may not have recognised each other as siblings because they were raised separately, one in captivity and one in the wild. However, the sibling mating between the two wild-hatched birds demonstrates that even siblings raised together in natural conditions will still sometimes mate.

The inbreeding levels found and the maximum and minimum levels estimated in this study (Table 3), are probably underestimated, as only full sibling matings are considered, and therefore, actual inbreeding levels are probably somewhat higher. Genetic analysis would be able to determine whether inbreeding between birds less related than full siblings (e.g. cousins) is occurring and if so, the extent of the inbreeding. However, some level of inbreeding is unavoidable given the small size of the founding population (about 23 adults before management commenced (Maloney & Murray, in press)), and therefore, this question is of more academic than applied interest.

The low level of close inbreeding is possibly a result of recognition of siblings and subsequent discrimination against them in the choice of mate. It is not achieved by dispersal as kākī do not disperse over large distances and congregate on river deltas prior to the breeding season. A comparison of the observed and expected levels of inbreeding would make it clear whether released birds are avoiding siblings as mates. If the observed level of inbreeding (Tables 2 & 3) is possible by chance, given the birds available in the area at the time of pair bonding, kākī are probably not actively avoiding sibling matings. If this is the case, more active management may be required if it is deemed necessary to prevent close inbreeding. If kākī mate with siblings less often than would be expected by chance, they are probably avoiding choosing siblings as mates. In this case, active management to prevent sibling matings may not be necessary. This comparison should be possible in the future as birds with unknown parentage die and are replaced with released birds of known parentage.

The tendency for birds to preferentially associate with siblings whom they were raised with (Table 5) indicates that learning of kin probably occurs primarily by association. If
recognition by phenotype matching also occurs, the lower average P value for cage-mates than siblings (Table 5) indicates that recognition by association is probably stronger. The reduced tendency to associate with siblings and cage-mates indicated by the generally higher P values three and seven months after the release of juveniles and sub-adults may in the case of the sub-adults reflect prospecting for mates and in both cases may be an artefact of the small sample. The lack of significant associations between siblings, sibling cage-mates and cage-mates when all five releases were averaged may be due to failure of the observers to correctly identify groups, rather than failure of birds to associate in groups. The congregation of released birds at food plates may have hindered group recognition in the observations one month post-release. Cage experiments would enable kin recognition in kaki to be better understood. Kin recognition in kaki chicks could be experimentally tested in captivity by recording the time spent in proximity to kin or non-kin. However, it would be necessary to use chicks less than one month old in order to avoid the risk of birds damaging themselves on the wire of cages (R. Maloney, pers. comm.).

The low level of inbreeding between full sibling released birds may be due in part or whole to the low survival rates of released birds between 1993 and 1998 (Figure 2). Now that the addition of iodine to the captive diet and post-release supplementary feeding has increased released bird survival (Maloney & Murray, in press), the number of sibling pairs may increase, although the proportion of inbred and outbred pairs should not change. Inbreeding levels in released and wild-hatched birds may also have remained similar because only 38% of released birds have been raised separately to siblings and 33% have been raised with unrelated cage-mates (Table 4). If these proportions increase, it is possible that the proportion of sibling pairs will also increase, as more siblings and less unrelated birds will be considered available as mates. For this reason it would be useful to keep a record of the relationship between all pairs and to summarise the data on a regular basis. In this way any tendencies for inbreeding to increase in years to come will be detected.

Of equal importance to the level of inbreeding is the effect of inbreeding on both reproductive success and survival. The number of sibling matings found in this study is too small to show whether inbreeding effects reproductive success and survival and it is too soon to know the outcomes of the 2000 inbred mating. If further matings between siblings occur, it would be worthwhile comparing reproductive success and survival of inbred and non-inbred offspring.

The known level of close inbreeding in both captive-reared and wild-hatched kaki and dark hybrids is much lower than that found in other New Zealand species with similar mating systems (e.g. from >8% of pairs to >50% of pairs of saddleback, Craig & Jenkins,
unpublished; tui, Stewart, 1980; blue duck, Williams, 1991 and Triggs et al., 1992); although the maximum possible inbreeding level is not (Table 3). It is probably not very useful to compare inbreeding levels in kākī with inbreeding levels in pukeko (Craig & Jamieson, 1988) as they have a different mating system and regularly mate with close relatives. Given that kākī and dark hybrids appear to have naturally low levels of inbreeding, the implication is that an increase in inbreeding is likely to cause negative effects. It is possible that the low survival rate in adult kākī, particularly females (Maloney & Murray, in press) is a result of past inbreeding depression. If this is the case, an increase in inbreeding caused by captive-rearing and release management could result in even more severe inbreeding depression and reduced survival. However, as the kākī population has been small for some time it is possible that even low levels of inbreeding may have purged at least some deleterious recessive alleles (Part, 1996; Pusey & Wolf, 1996). If this is the case, a management-induced increase in inbreeding may not result in reduced survival or reproductive success. Any beneficial effects of inbreeding (Templeton, 1986; Butler & Merton, 1992) are likely to have already accrued in the population, given it’s history of small size, so it is not likely that further inbreeding will bring about further significant biological or management benefits.

In the absence of data about whether inbreeding reduces survival or reproductive success in kākī, it would seem prudent to minimise potential inbreeding (also see Wallis, 1999). This could be approached from two angles. While some separation of siblings at the aviary is inevitable given multiple clutching, the separation of clutches and mixing of unrelated birds at the aviary should be minimised as far as possible. Rather than releasing several sibling broods into one area, multiple broods from a single breeding pair could be distributed between the sub-populations (Figure 3). In the long term, multiple broods could be spread over the Mackenzie Basin population and any new population that was established elsewhere. However, releasing smaller numbers of birds into several areas is contrary to the principle that rapidly increasing population size minimises the risk of extinction (Frankel & Soule, 1981) and the management aim of providing the maximum number of potential mates to minimise the risk of hybridisation (Maloney & Murray, in press). The risk of inbreeding increasing if unknown siblings are released into the same area must be weighed up against the risk of hybridisation because of insufficient mate availability if unknown siblings are released into different areas. However, the increased survival since 1998 (Maloney & Murray, in press) means that insufficient mate availability is now less likely to be limiting. Releasing several smaller groups into several areas would also minimise possible problems of overcrowding, which were suspected to have caused the disappearance of released juveniles following the February 2000 release into the Cass River (Neill et al., 2001). Minimising inbreeding in released
birds is also advisable given the long-term aim of establishing kākā throughout New Zealand (Maloney & Murray, in press), as inbred populations may have lower tolerance to environmental stress and changes in environmental conditions (e.g. Keller et al., 1994; Jamieson & Ryan, 2000).

While Sanders (1996) found that most kākā spend their lives within one geographic sub-population range, there are occasional movements between sub-populations. Even if inbreeding levels do increase in the future as a result of captive-rearing and release management, the occasional migration of birds between sub-populations provides a source of genetic variation. Even one successful migrant between groups may be sufficient to prevent homozygosity building up (Ralls et al., 1986), hence greater inbreeding may not result in any loss of genetic variety at the population level provided birds continue to occasionally move between sub-populations. There is no reason to believe that such movements will not continue.

Whether or not close inbreeding can be minimised, and genetic variety maintained at current levels, is secondary in importance to the recovery of the species. The first priority of kākā management is to increase numbers in the wild (Maloney & Murray, in press). As numbers of breeding pairs in the wild increase as a result of multiple clutching and release management, the ability to raise multiple clutches from each pair will be reduced due to finite aviary capacity. Either more aviary space will be needed, or managers will need to decide whether to raise multiple clutches from a smaller number of pairs, or fewer clutches from a larger number of pairs. The logical choice would appear to be raising fewer clutches from a larger number of pairs. The eventual reduction in multiple clutching will in turn reduce the likelihood of full sibling matings. Eventually the population should recover to the point where it is no longer necessary to multiple clutch and the possibility that management could increase sibling matings will no longer be an issue. The possibility of a short-term increase in the likelihood of sibling matings brought about by multiple clutching may therefore be justifiable in light of a long-term recovery of the species.
CONCLUSIONS AND RECOMMENDATIONS

Close inbreeding does not currently appear to be a problem for captive-reared released kaki. However, I recommend a precautionary approach, as there is a possibility that close inbreeding will increase in the future as a result of improved survival and multiple clutching or clutch intermingling and close inbreeding commonly results in negative effects. It would be useful to perform kin recognition tests with chicks to further investigate the possibility that kaki view siblings with whom they have not been raised as potential mates and unrelated birds with whom they have been raised as siblings and unavailable. While a certain amount of inbreeding is inevitable given kaki's history, in view of the indication that kaki learn kin through association, the opportunity for close inbreeding should be minimised where possible and inbreeding levels should be monitored.

The possibility of close inbreeding may be minimised by aviary and release management. Raising of kaki separately to siblings and with unrelated cage-mates should be minimised where possible. Releases could distribute siblings that were not raised together over different sub-populations to avoid kaki coming into contact with siblings they may consider to be potential mates. Due to increased survival, mate availability is now less likely to be limiting, removing the necessity of large releases into a single area.

However, even if sibling matings do increase, it is likely that movement between sub-populations will be frequent enough to maintain genetic variability at current levels on a population scale. Whether or not this is the case, a short-term increase in close inbreeding is tolerable if it results in recovery of the species.
REFERENCES

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