

# Genetic status of New Zealand black stilt (*Himantopus novaezelandiae*) and impact of hybridisation

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# Abstract

The New Zealand black stilt, *Himantopus novaezelandiae*, is critically endangered, with only 67 "pure" birds remaining, all in the upper Waitaki Basin. Hybridisation with pied stilt, *H. leucocephalus*, is compounding problems caused by lack of habitat and predation. This report looks at the likely impact and implications of this hybridisation from a genetical perspective. Based on morphological, genetical and phylogenetic data, it is concluded that the two taxa merit recognition as distinct species under most species concepts. With reference to plumage characteristics alone, it appears that females with a degree of hybridity show reduced fitness with respect to the fledging success and survival of their offspring. Simulations of gene flow from pied into black stilts, using a simple single locus two allele model, suggest that reduced fitness in hybrids may be sufficient to counteract low levels of gene flow, preventing genetic swamping of black stilt in the short term at least. A key unknown factor here is the degree of assortative mating under different population sizes and densities. Based on these findings, several management recommendations are made, including: 1) to carry out multivariate analysis of morphological data to determine the best estimators of hybridity; 2) to use these morphological characters as well as plumage to measure hybridity of birds used for captive rearing; 3) to determine the heritability of these traits; and 4) to carry out a more detailed analysis of outbreeding depression using pedigree information. As a general conclusion, although there are genetical threats to the species, the main issues are, as usual, habitat loss and predation, and the associated stochastic problems of small population size.

## 1. Background

New Zealand is home to two species of stilt: pied (poaka; *Himantopus himantopus leucocephalus*) and black (kaki; *H. novaezelandiae*). These are thought to derive from two separate colonisation events by pied stilt from Australia: one recent and the other ancient. Breeding kaki are now confined to the hydro lakes of the Mackenzie Basin (Pukaki, Benmore, Ohau, Tekapo) and the intervening river beds and associated wetlands (Maloney *et al.* 1997), but records show that they were previously much more widespread in South Island (Holdaway 1995; Pierce 1984a). In contrast, pied stilts have expanded their range inland since their arrival, perhaps as recently as 150 years ago. Habitat modification of braided river beds has impacted negatively on all waders, but pied stilts appear to have broader habitat requirements than kaki and have not been so affected (Robertson *et al.* 1983). Kaki appear to prefer faster flowing water and may suffer more from introduced predators (Pierce 1982; Pierce 1986; Reed 1987; Robertson *et al.* 1983).

Mammalian predators (cat, rat, mustelid, hedgehog) in the Mackenzie basin threaten the severely reduced kaki population further. Rabbit control by 1080 drops and RCD may exacerbate the situation by forcing predators to switch

from rabbits to birds. In general, any population of this size is under severe threat from a number of potential extinction vortices (Gilpin & Soule 1986).

In addition to these problems, the integrity of the kaki gene pool is threatened by introgression from the pied stilt. During the spring, kaki are greatly outnumbered by peds, and hybridisation has been documented for many years. This threat would undoubtedly be much greater if it were not for the fact that kaki are largely resident through the winter and tend to form intraspecific pair ponds before the migratory peds return in the spring.

The purpose of this report is to focus specifically on the genetic status and threats to the remaining kaki of the Mackenzie Basin, with particular reference to intraspecific hybridisation, and to make some genetical research and management recommendations.

## 2. Current status and management

The Mackenzie population of kaki currently comprises 67 "pure" black birds and 31 dark hybrids with primarily kaki ancestry. In the 1997-98 breeding season there were 16 pairs of birds (black and hybrid) breeding throughout the Mackenzie Basin. Low population numbers, restricted distribution, habitat and predation threat and ecological displacement by peds have led to their Category A listing (Molloy & Davis 1994).

Since 1982, all matings involving kaki have been monitored. Matings with peds have been disrupted to encourage intraspecific matings. Since 1987, all eggs from kaki intraspecific matings have been removed, replaced by dummy eggs, and hatched in captivity (Reed 1998). Returned chicks have shown low survival, probably because of predation. More than half of the eggs produced annually are hatched and reared to nine months old in cages. Chicks are now reared in cages, and released back into their parents' breeding ground, which is proving a more successful strategy.

## 3. Genetical considerations

### 3.1 SPECIES STATUS

One of the first questions one might ask about kaki is: what is its specific status? Systematists have suggested at least 23 published species concepts (Appendix 1), but in reality only a few of these are widely considered: morphological, biological, recognition, phylogenetic and concordance species concepts. Systematists defining species on the basis of differences in pelage or plumage colour, particularly in the case of dark varieties, have often been

proved wrong in the past (Aulsebrook, 1989). This is of particular relevance in the conservation of New Zealand birds. Some species (e.g. fantail) show dark and typical morphs within the same population; other species (variable oystercatcher, little shag) show geographic variation, with higher proportions of darker morphs to the south. In these species, the morphs are regarded as conspecifics. Melanic forms of other species (black robin, kaki), however, are recognised as species in their own right. What is the evidence for the specific status of kaki under these five species concepts?

### **Morphology**

Under a classical morphological species concept, there is little doubt that kaki merit species status. They have a longer and broader bill, longer wings and tail, and shorter tarsi than piers (Pierce 1984b), and the call is slightly higher. However, larger body size (Bergmann's rule), smaller extremities (Allen's rule), longer bills and longer wings are all predicted in colder climates (Mayr 1942), and the dark plumage (Gloger's rule) may also indicate physiological adaptation, so kaki could be interpreted as a cold-adapted ecotype.

### **Genetics**

The biological species concept (BSC) (Mayr 1940) emphasises both the potential for interbreeding among (conspecific) populations and reproductive isolation among species. The fact that kaki and piers can interbreed successfully questions the integrity of the species; indeed Mayr himself considered them subspecies (Mayr & Short 1970). However, one could allow for a small amount of interbreeding among species; long-term persistence of two forms in the face of a little gene flow is generally considered permissible (Arntzen & Wallis 1991). Additionally, hybridisation often tends to increase when one form is rare; i.e. the current level of hybridisation may be artificially high as a result of the small population size of kaki. Green's (1988) isozyme analyses strongly suggest that NZ piers contain introgressed kaki genes: their gene frequencies are intermediate between Australian piers and kaki and they have higher heterozygosity. This genetic observation is in keeping with the intermediacy in plumage characteristics, that is, all NZ piers are darker than Australian piers (Pierce 1984b).

The recognition concept of species (Paterson 1985) is similar to the biological species concept in that it emphasises conspecific populations as the field for gene exchange, but it seeks to define species purely in these terms, i.e. in terms of shared fertilisation systems. Any two taxa that regularly interbreed are by definition conspecifics, irrespective of post-zygotic isolating effects. Under this concept, stilts would be seen as conspecific. However, like the BSC, one could allow for a little breakdown in the specificity of the mate recognition system, especially in the case of modified habitat. The recognition species concept has not been widely accepted (Coyne et al. 1988), and is particularly open to question in the case of hybridising taxa that may have deep independent phylogenetic histories.

## Phylogenetics

The general acceptance of phylogenetic principles in taxonomy and the development of phylogenetic methodology and molecular techniques has led to various formulations of a phylogenetic species concept (PSC, Cracraft, 1983; McKittrick and Zink, 1988). The important features of the PSC are monophyly and diagnosability. The mtDNA sequence data are relevant in this regard (MacAvoy and Chambers, 1997). Data for the control region, a relatively fast evolving sequence, resolve two major haplotype groups with 16 Australian and NZ pied in one clade, and nine kaki in the other. If we were dealing with a single polymorphic population, the chance of 25 haplotypes partitioning themselves in this way across the two taxa is less than  $5 \times 10^{-7}$ ; therefore we are dealing with two independent gene pools. There is also a single kaki that possesses the pied-type, but this bird comes from a J x I mating, so is known to have hybrid origins. Of four other hybrids analysed, two fall into each category. Sequence data for *cytb* also resolve two concordant haplotype groups. The two major haplotype groups differ by 17-18 bases. There are no "intermediate" haplotypes, indicating a considerable period of independent evolution of the two forms so that there has been complete lineage sorting (Avice and Ball, 1990) and molecular evolution of the mitochondrial genome in kaki. So under the PSC there are two species.

## Concordance

Kaki and pied stilt also conform to species under the concordance species concept (Avice and Ball, 1990). This concept of a species has its base in the biological species concept, but emphasises the significance of repeated pattern among diagnostic markers. In the case of stilts, morphological, plumage, isozyme and mtDNA markers concord with each other, reinforcing their species status.

## Genetic distance

Some workers have sought to infer species status from genetic distance values derived from isozyme studies, but this practice is problematic because time since divergence is decoupled from reproductive isolation, and there are numerous different calibrations used (Avice & Aquadro 1982). Interspecific D values in birds are often very small, so the D value of 0.006 - 0.01 (Green 1988) reported for kaki vs pied does not mean much *per se*, except that the species are not separated by very much evolutionary time, although recent gene flow may have merged the two gene pools over the last century. The mtDNA control region data are more informative: kaki show about 5% sequence divergence from both Australian and NZ pied, which are indistinguishable from one another. This amount of divergence is typical for closely related or sibling bird species (Kidd & Friesen 1998; Marshall & Baker 1997; Merila *et al.* 1997; Wenink *et al.* 1994) and probably represents around one million years of separation. Hence kaki may be a Pleistocene relict, which became adapted to the periglacial grasslands that dominated at the time.

## **Evolutionarily significant units and management units**

For conservation purposes, emphasis has moved away from species concepts and more towards defining evolutionarily significant units (ESUs) and management units (MUs). Moritz has suggested that ESUs should be reciprocally monophyletic for mtDNA haplotypes and possess a frequency difference for at least one nuclear gene (Moritz 1994). A unit that has only the latter property is defined as an MU. Aside from the recent introgression, kaki and pied are clearly reciprocally monophyletic groups.

### **3.2 WHAT ARE WE TRYING TO CONSERVE?**

The philosophical issue of what we are trying to conserve by management has always been around, but the issue becomes bigger with the application of genetical techniques. Are we trying to conserve a population? A species? Phenotype? Behaviour? Composite genotype? A lineage? As many of those things as possible? As introgression has already taken place, "purity" of the species may be a lost cause.

When we think of conserving biodiversity at a genetic level, we are really using a limited set of genes as indicators for the entire genome. If introgression has occurred, one requires a very large bank of loci to detect alien genes effectively. One suggestion might be to build a set of species specific nuclear markers (e.g. RAPDs and microsatellites) to cover the genome, then screen all remaining Node J birds to detect alien genes so that one can avoid using birds with more than a certain amount of pied ancestry. There is commercial demand for this approach in deer farming, for example, so that wapiti genes can be minimised in red deer herds. Not only is this suggestion impractical from a financial viewpoint, it may also remove beneficial genes from the gene pool (see section on outbreeding depression). Additionally, many of the black birds may have some hybrid ancestry, as attested by the fact that 35% of offspring from J x J crosses are hybrid nodes (Table 1).

Mitochondrial haplotype is completely decoupled from the nuclear genome. There are many examples of mitochondrial genomes crossing species boundaries (Avice 1994), particularly in association with hybrid zones. The opposite has been observed also, where isozymes are very similar between two hybridising flycatchers, but the mtDNAs remain quite distinct (Tegelstrom and Gelter, 1990). This observation can be explained by male-mediated nuclear gene flow with hybrid female sterility according to Haldane's rule. A similar case may apply in kaki and pied stilt. The main point is that the mitochondrial genome is a maternally inherited non-recombining genome, and merely indicates the cytoplasmic ancestry of a bird. The mitochondrial genome (16000bp) is less than 0.001% of the size of the nuclear genome, so is not of much relevance if we are considering quantifying degree of hybridity, although it is useful for detecting ancient hybridisation.

The value of kaki lies primarily in its morphological and behavioural distinctiveness, and this distinctiveness derives phylogenetic substance from the divergence observed in the mitochondrial sequences (MacAvoy & Chambers

1997). The current policy of only selecting dark plumage birds as parents is sensible, but not sufficient in itself. Morphological characters (bill, wings, tail, tarsus) should be checked in potential breeders, because many plumage genes will be unlinked to other morphological markers. As kaki currently have a degree of hybridity, there is a danger of losing the identity of the taxon for these other characteristics if plumage alone is used to select parents. Additionally, hybrid matings could be restricted to J x I and J x H as these already provide 93% of breeding birds for the next generation.

An interesting potential parallel to kaki is the now-extinct dusky seaside sparrow (*Ammodramus maritimus nigrescens*), which was the centre of a major conservation effort in the US some 15 years ago. The dusky was one of nine named subspecies of seaside sparrow in the coastal eastern and southern US and by 1970 was restricted to a single population in Florida. mtDNA analysis of these nine forms revealed a significant binary split between the five Gulf coast subspecies and the four Atlantic coast subspecies, but revealed no genetic basis for recognition of the melanic form as distinct from the other Atlantic coast forms (Avisé & Nelson 1989). However, in contrast to the dusky seaside sparrow, NZ kaki are quite distinct from peds for a number of features, and represent something much more significant than merely a melanic form.

### 3.3 GENETIC CONTROL OF PLUMAGE CHARACTERISTICS

The mechanism of genetic control of plumage characteristics would probably be complex to unravel without extensive mating experiments. However, we can make some statements based on the many crosses that have been made.

Rather than being discrete classes, the 12 "nodes" of plumage are convenient points on a continuum for classification of birds (Pierce 1984b). Plumage is likely to be a quantitative or polygenic trait with some environmental influence. Not all alleles will be of equal effect, and their effects may not simply be additive, within or among loci. The intergradation of morphs can be explained either by multiple loci segregating variation with an effect on the trait, or an unknown number of loci with an environmental influence overlain. If the environmental effect is large, then only a very small number of loci need be invoked to explain the continuity of morph nodes. However, as peds and kaki appear to breed true for their plumage, environmental modulation is probably relatively minor, implying several loci influencing the trait.

An interesting feature is that J x J crosses can produce some intermediate plumage types (Table t ; 65% J, 20% I, 10% H, 5% G; N = 60). This observation suggests an element of recessivity in the pied plumage characters. Similarly, the J x I crosses show some H plumage types (61% J, 31% I, 8% H; N = 13) and J x H crosses show some G plumage types (33% J, 17% I, 33% H, 17% G; N = 6). Data for offspring reaching 2 years are only available for six other offspring across three types of cross. The five types of J x hybrid cross as a whole produce about 50% more hybrid types than the J x J crosses (45% J, 27% I, 18% H, 9% G; N = 22).



Statistical analysis is difficult given the small sample sizes (only 84/1025 (8.2%) of eggs produce birds that survive to 2 years), the non-independence of the data, and the large array of possible genetical models.

An alternative explanation for the occasional offspring node outside of the parental range, which has been ignored above, is that of extra-pair paternity (EPP). EP copulations are seen, especially in young birds, and are well-established in other waders. If we are unable to rule out EPP, then such segregants may not really exist.

### 3.4 HYBRIDISATION

Hybridisation is a fascinating and useful phenomenon for evolutionary biologists, but it creates problems for systematists and conservation biologists alike: it blurs the neat groupings that both would like and compromises phylogenetic integrity. Literal interpretation of the US Endangered Species Act of 1973 provided loopholes for various land-use agencies to petition for removal of endangered taxa with hybrid ancestry from the list: red wolf and Florida panther were two high-profile species threatened in this way. Pressure was brought to bear to change this "hybrid policy" (O'Brien & Mayr 1991), which has now been suspended indefinitely.

One feature of hybridisation that should be considered in conservation is its "naturalness". Occasional hybridisation among sympatric species, or stable hybrid zones, are common natural phenomena. Examples of hybrid zones in birds are less common, probably because of their mobility. If hybrid zones are best seen as tension zones maintained by a balance between dispersal (widening zone) and selection against hybrids (narrowing zone), then we would expect to see them less frequently in mobile species. Some classic bird examples include the ring species (Artenkreis) of Arctic gulls, and hybrid zones between European crows, Scandinavian flycatchers and North American flickers, wood warblers, sapsuckers, ducks and crowned sparrows.

However, many cases of hybridisation result from recent habitat modification or introduction of related alien species. Introduced mallard are interbreeding with and replacing native grey duck (Hitchmough *et al.* 1990; Rhymer *et al.*, 1994), black robin/tomtit hybrids have been produced as a result of mis-imprinting through the cross-fostering programme (Lambert, pers. comm.); black browed mollymawk subspecies are hybridising on Campbell Island as the result of a small natural introduction (Avis 1995); the Chatham's endemic Forbes parakeet is threatened by introgressive hybridisation with red-crowned parakeet as a result of forest reduction (Triggs & Daugherty 1988); yellow-crowned and red-crowned parakeets have almost totally merged on the Auckland Islands (Avis 1995).

Where do kākā fit into the picture? Clearly the pied stilt introduction was natural, but their subsequent rapid range expansion at the expense of kākā is undoubtedly in large part due to habitat modification through land clearance and river management, and following introduction of mammalian predators. Thus, they appear to fit into recommendation 4 in a DOC discussion paper on management of hybridisation:

"Where hybridisation is occurring and human impacts have been implicated and either species is threatened, if the technology exists to mitigate the threat the Department should be obliged to do so." (Avis 1995).

### 3.5 OUTBREEDING DEPRESSION

A common feature of hybridisation between species is some overdominance ("hybrid vigour") in the F<sub>1</sub>, followed by outbreeding depression ("hybrid breakdown") in the F<sub>2</sub> (Harrison, 1993). Outbreeding depression can be intrinsic (e.g. a result of chromosome or gene incompatibilities, or developmental failure) or extrinsic (e.g. lack of environmental fit or behavioural incompatibilities of intermediates). It may range through: F<sub>1</sub> inviability, F<sub>1</sub> sterility, reduced F<sub>1</sub> fitness, reduced mean viability of F<sub>2</sub>. To the conservation biologist, therefore, there is not only the threat of loss of integrity of the introgressed form, but the threat of long-term loss of fitness as a direct result of hybridity. This fitness loss, however, is often restricted to one of the sexes. Haldane's Rule states that whenever one sex is absent, rare, or sterile, it is the heterogametic sex (XY males in mammals; ZW females in birds). This law is one of the most enduring in evolutionary biology. A recent review of interspecific hybridisation in birds showed that where one sex is inviable it is nearly always the female (21/23), and where one sex is sterile, it is always the female (30/30) (Wu *et al.* 1996).

With one exception (J x H 2yr/egg), fledgling and 2-year (reproductive age) survivor rates, both per egg and per hatchling, are lower in matings involving nodes F, G, and H as one of the parents than they are in J x I or J x J matings (Table 1). The difference may be as much as 2 to 3-fold for some comparisons. Testing for heterogeneity among the five matings Q x J to J x F inclusive) in the four fecundity measures (eggs to 2 yr inclusive) using a heterogeneity G-test (Sokal & Rohlf 1981) gives a value that is not quite significant at the 5 level ( $G_{H(12)} = 18.9$ ;  $0.1 > P > 0.05$ ); that is, there is little evidence of heterogeneity for fecundity among mating types. Friedman's test for randomised blocks using rankings (1-5) for the four central fitness measures (fledge/egg to 2 yr/hatch inclusive) gives a value significant at the 2.5% level ( $\chi^2_{(4)} = 12.6$ ;  $0.025 > P > 0.01$ ), indicating that there is consistency in the fitness rankings across the types of measure used. As there is some variation in management practice over time, and this could affect fecundity, the data were reanalysed including only birds from 1987 onwards, when there was a change away from cross-fostering and towards the current egg management regime. The reduced dataset gives  $G = 20.64$ ;  $0.1 > P > 0.05$ . Hence there is no evidence for an effect of altered management.

The matings have been divided into those involving a hybrid male and those involving a hybrid female (Table 2). As predicted by Haldane's rule, there is a reduction in hatching and fledging success of offspring from female hybrid nodes F, G, and H, but no discernible effect in males. The same G-test now gives a very low value for male heterogeneity ( $G_{H(6)} = 2.21$ ;  $P = 0.9$ ) but a highly significant value for females ( $G_{H(6)} = 27.6$ ;  $P < 0.001$ ).

The caveats here involve the lack of independence of the data (i.e. females have multiple offspring/broods) and the current lack of data on survival to 2

years where the sex of the parent nodes is known. Another inconsistent feature of the data is the 100% hatching success (28/28) of F females compared with 12.9% (4/31) in G females, suggesting that there are other factors involved in this parameter of fitness. This aspect of hybridisation is worth quantifying more carefully, since it is critical in the simulation of genetic mixing, and there are management implications.

### 3.6 INBREEDING DEPRESSION

Inbreeding is the bringing together of genes that are identical by descent through the matings of related individuals. In the short-term, inbreeding reduces heterozygosity and reduces effective population number. The long-term effect of small population size includes loss of genetic variability. Thus, in a species that normally outbreeds, inbreeding will almost certainly have a negative impact (Keller *et al.* 1994; Saccheri *et al.* 1998). However, the extent to which inbreeding threatens the continued existence of a species has been widely debated (Wallis 1994). In general, it is fair to say that other factors such as loss of habitat and predation are of much more significance to the probability of long-term survival than inbreeding depression. Species are generally much more likely to enter an extinction vortex as a result of demographic factors rather than genetical ones (Lande 1988). With respect to captive-reared birds, though, inbreeding should either be avoided or minimised. Guidelines for situations where inbreeding cannot be avoided have been suggested (Templeton & Read 1984). In these instances, attempts should be made to increase population size as quickly as possible, to avoid any sudden increase in inbreeding coefficient, and ensure that founders are all represented (if not equally) in descendants.

### 3.7 GENE FLOW

Gene flow of neutral alleles among taxa can quickly lead to homogenisation of those taxa in a hybrid swarm. Population genetics theory shows that a single migrant per generation is enough to prevent genetic divergence by drift, irrespective of population size. Any more than this amount will result in the merging of two genetically distinct populations. Selection against alien alleles can counteract this homogenisation process, if the ratio of selection coefficient to migration rate is high. Narrow hybrid zones can be maintained in the face of gene flow if dispersal is low, and/or selection against hybrids is high (Harrison 1993). Habitat preference across an ecotone will serve to stabilise the situation considerably.

Hybridisation in NZ stilts does not conform to classical hybrid zone models: introgression is heavily one-sided (black being absorbed by pied), there is wholesale replacement of kaki by pied, and the species are geographically mixed. In ecological terms, they still show demographic exchangeability (Templeton 1989). Apart from habitat and predator problems, the gene flow resulting from just a few interspecific matings per generation does not appear to bode well for the future of kaki, without some sort of intensive management.

We can simulate the effects of gene flow with a fairly simple model using PopGen 2.0 (Wells, 1992). Assumptions are that the source population (pieds) remain genetically stable (since they outnumber kaki by about ten-fold in Mackenzie Basin alone), that the NZ pied genome is in reality about 15% kaki in origin (based on plumage), and that the kaki genome is about 6% pied (based on 1995/96 breeding report). The model considers allele frequency at a single mendelian locus with two alleles: one pied type and one kaki type. Since 30% of kaki paired with pieds last season, then the rate of gene flow ( $m$ ) into the kaki population was set at 15%, at the high end of the range. Rates that were up to an order of magnitude smaller than this rate were also modelled. Based on the data from hybrid crosses, more intermediate node hybrids have a fitness ( $w$ ) of only 50% that of kaki. Eight independent replicates of all simulations were run for 50 generations.

The results show that, under current estimated parameter values (Fig. 1a;  $m = 15\%$ ;  $w = 50\%$ ), pied genes would make up about 50% of the population after 10 generations, and would totally swamp kaki before 20 generations elapsed. If, however,  $m$  is reduced to 5% (Fig. 1b), kaki maintain their integrity indefinitely. What is happening is that the reduced fitness of hybrids is overcoming the process of gene flow.

Conservatively, as the darker node intermediates may not show much reduction in fitness, simulations were carried out where fitness ( $w$ ) of hybrids is 75% and 90% that of homozygotes. In the first case, as before, 15% migration quickly swamps the kaki gene pool (Fig. 2a). Reducing  $m$  to 5% merely slows down the process, so that a 50% mixed population will on average prevail by about 40 generations (Fig. 2b). It is only by reducing  $m$  to 3% that the integrity of the kaki population is maintained, and even then, drift can occasionally take the population over the threshold where selection no longer works effectively against alien genes as they start to occur in homozygous form (Fig. 2c). If the fitness of hybrids is as high as 90% that of homozygotes, then migration of 5% will give a 50% mixed population on average by about 25 generations (Fig. 3a). Reducing  $m$  to 3% now has little effect on slowing this process (Fig. 3b);  $m$  must be reduced to less than 1% to maintain integrity of the kaki gene pool for 50 generations, but frequencies of pied genes will have drifted significantly higher over this period (Fig. 3c). While the pied population in the region remains large, the long-term (i.e. centuries) prediction under all of these scenarios is a slow drift towards pied morphology.

There are several caveats to these simulations: (1) they are based on a single locus polymorphism - the trait is probably influenced by many loci of varying individual effect; (2) hybrid fitness is considered constant and intrinsic; (3) Male and female fitnesses are not distinguished. Using various parameter values to some extent compensates for this uncertainty.

### 3.8 ASSORTATIVE MATING

A key factor in the applicability of the simulations above is the degree to which the strength of assortative mating correlates with increasing population size, and that is something that we have no idea of. The fact that 70% of blacks pair with another black indicates quite strong assortative mating given

that they only make up 3% of the total stilt population (Pierce 1982; Pierce 1984b). This behaviour could purely be an effect of the absence of migratory behaviour in kaki rather than mate choice. While we might expect a larger population of kaki to show even greater assortative mating, we cannot be certain that this will be so.

## 4. Conclusions

In accordance with earlier workers (Green, 1988; Pierce, 1982) I believe that kaki merit specific status. The mitochondrial DNA data reinforce this view and kaki clearly qualifies as an ESU. Although genetically similar to pied stilt, it is a unique component of NZ biodiversity. It might be helpful to increase the sample size of node J kaki examined for mtDNA to determine whether any of these birds show evidence of hybrid ancestry. This analysis should be done using RFLPs of the amplified control region (MacAvoy & Chambers 1997).

Rather than focus on genetic characteristics, which in the case of nuclear genes would be expensive to assess, and in the case of mtDNA is of limited value, birds for breeding should be chosen on the basis of both plumage and morphological attributes. It would be helpful to carry out some detailed analysis of the morphological variation in kaki, piers and hybrids using multivariate analysis. This work could be done as part of a student project. It would also be useful to look for published examples of genetic analysis of plumage characteristics in other birds.

A more thorough analysis of all pedigree data would help throw more light on both the genetic control of plumage and morphology. By examining offspring plumage and morphology by individual cross, rather than pooling across parental node, an estimate of heritability for the traits could be made. This approach involves fairly simple regression analysis of parents and offspring. This work could be done as part of a student project, and is more of academic than applied interest.

The reduction in fitness seen in hybrids tends to ameliorate the effects of gene flow from piers. It is not possible, however, to predict how a large population of kaki would behave in terms of assortative mating. Research on assortative mating in the wild is required, particularly its relationship with increased population size. If this can be shown to be strong, then it does seem likely that with a larger population size, gene flow would be minimised, and lead to a relatively stable situation (genetically at least) over many generations. In the long term, though, gradual introgression of neutral genes that do not reduce hybrid viability will occur.

A more thorough analysis of all pedigree data would help throw more light on hybrid breakdown. The analysis is currently compromised by lack of independence of the data because of the pooling of like nodes. To get a more reliable estimate of fitness depression, including distinguishing possible inbreeding effects from outbreeding depression, we need to examine fitness by

individual cross, rather than pooling across parental node. Also, data for survival to 2 years would be valuable for the analysis by sex. This work could be done as part of a student project, and is of applied significance.

Despite the caveats over the analysis of outbreeding depression, I would suggest that captive rearing should avoid using any females lighter than node I, given the likely reduced fitness of these birds.

If there are some hybrids of unknown parentage in the wild, it might be useful to determine mtDNA type in these birds. If the sample size were large, it might be possible to detect asymmetric hybridisation, if it were occurring. There is also the more academic question of whether kaki mtDNA can be found in piers, which would confirm Green's suggestion that pier have mixed ancestry. It is not clear that this work would provide answers of relevance to kaki management, though.

A general comment: The problems of hybridisation faced by kaki are very much the final assault on the species; they are an effect of the problem rather than a cause. Any successful rehabilitation of the species needs to focus on increasing the ratio of kaki to pier stilt in the region. This may require large-scale habitat change, wholesale removal of pier stilt from the region, predator control and monitoring, or some combination of all of these approaches.

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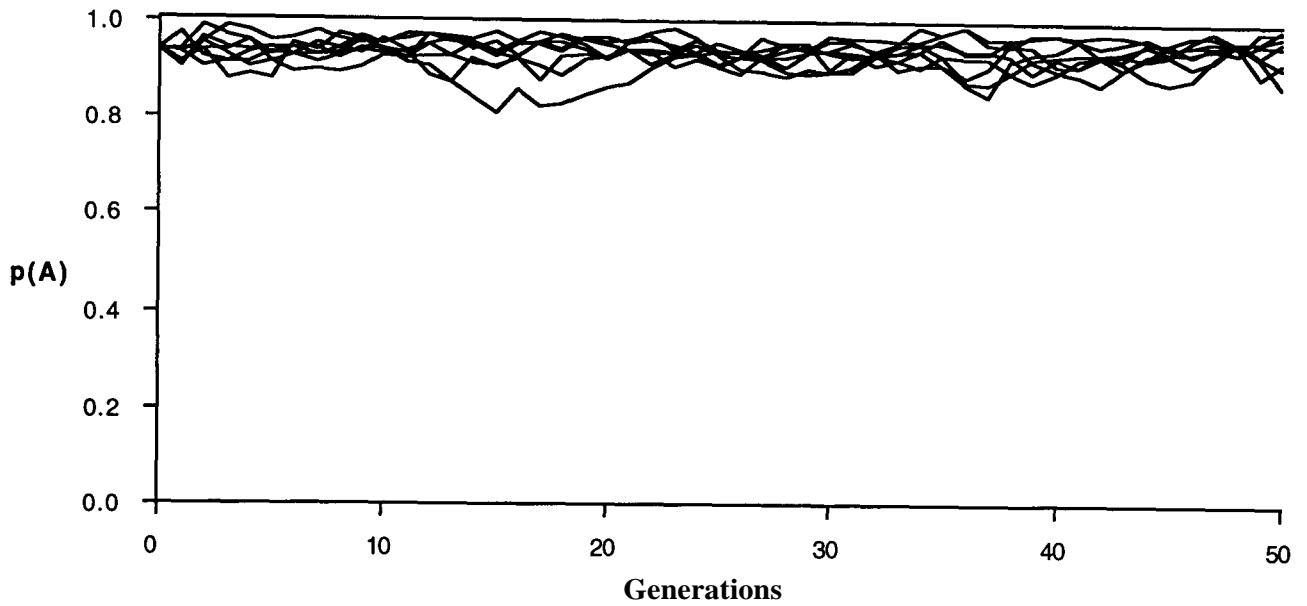
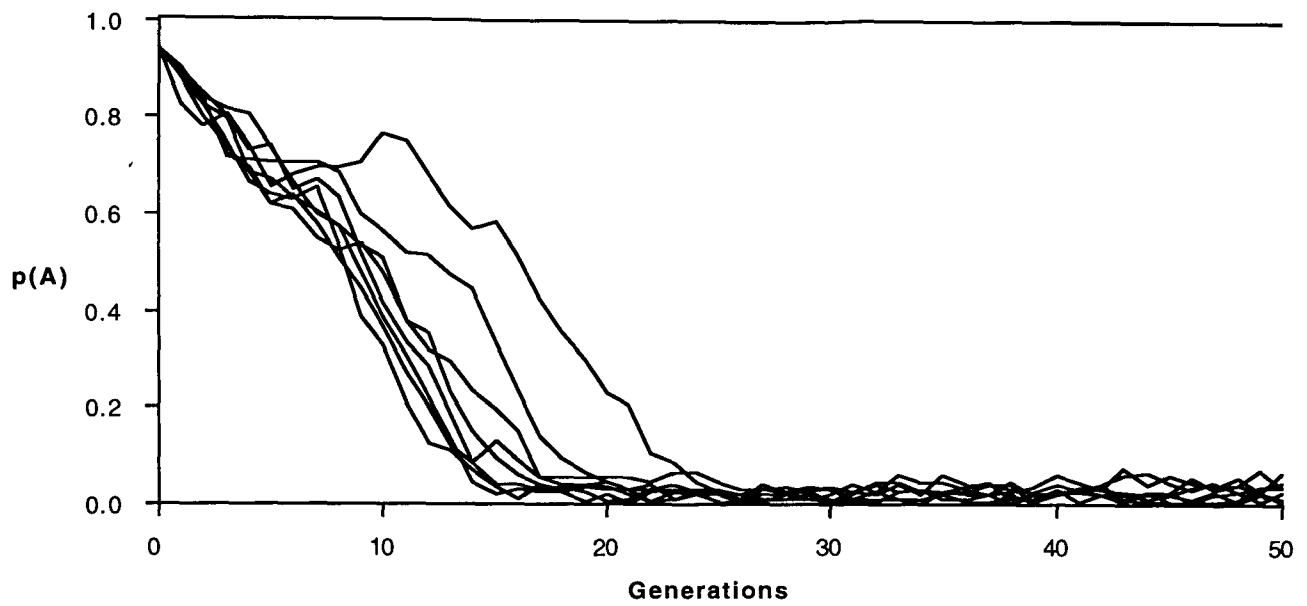
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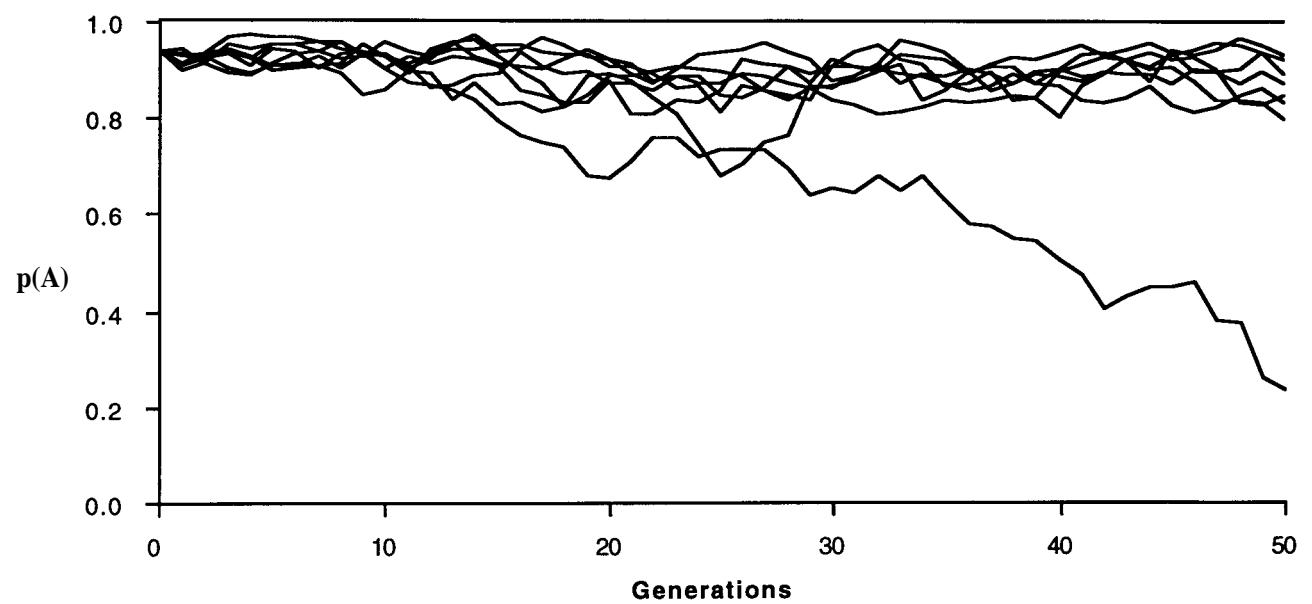
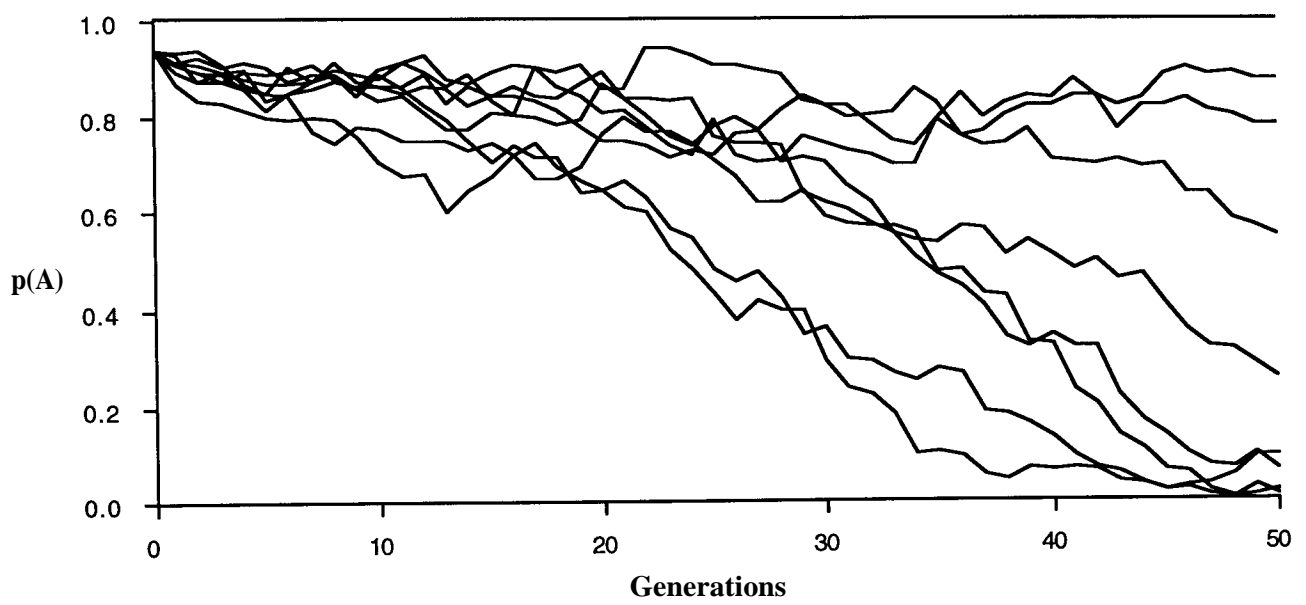
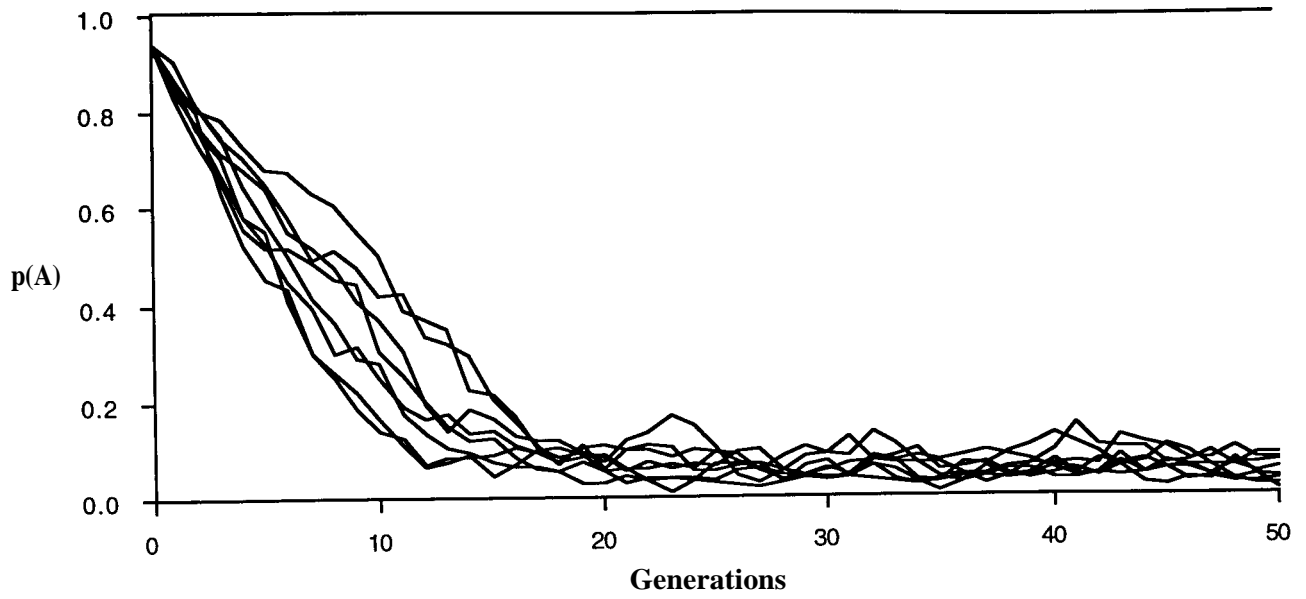
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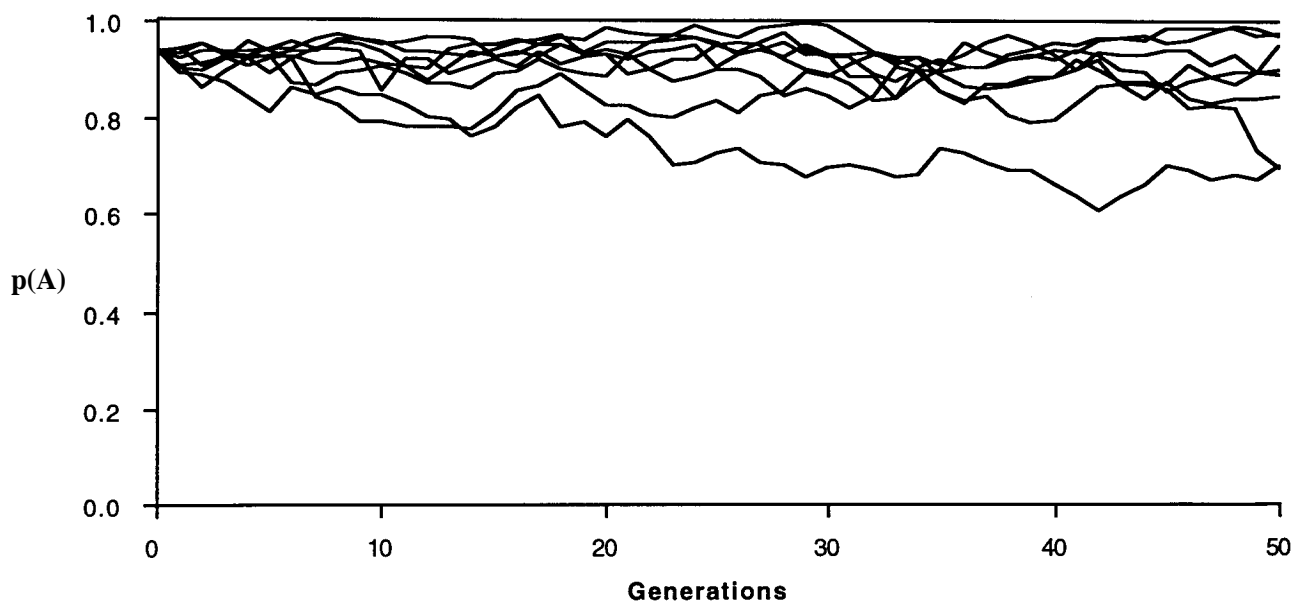
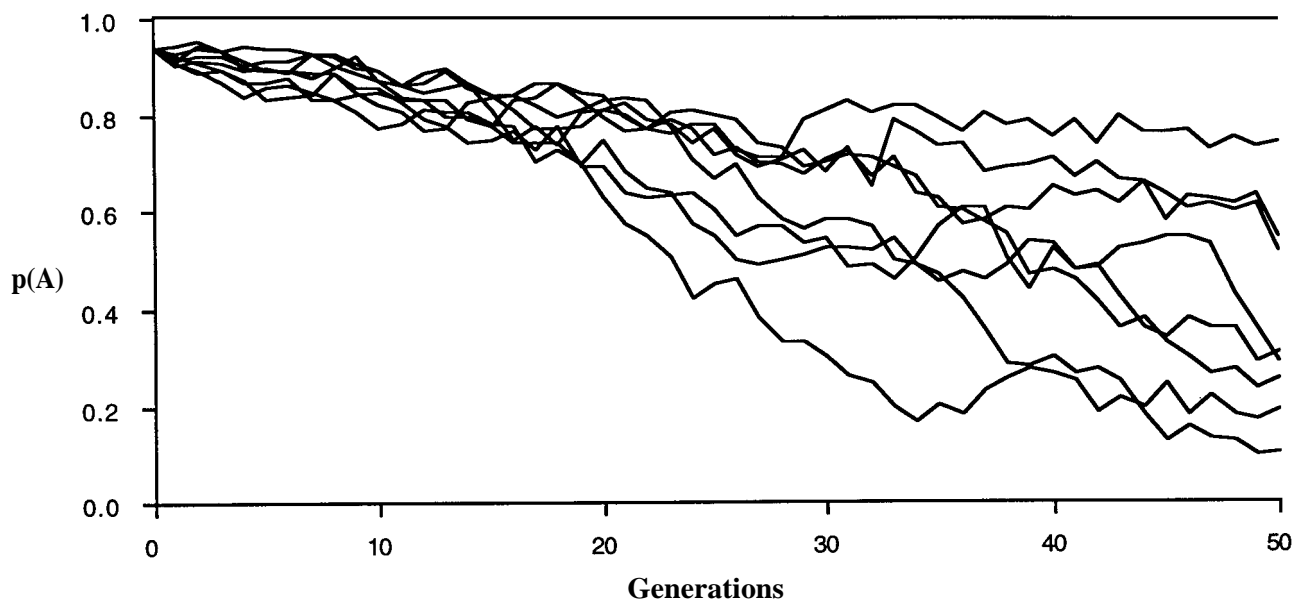
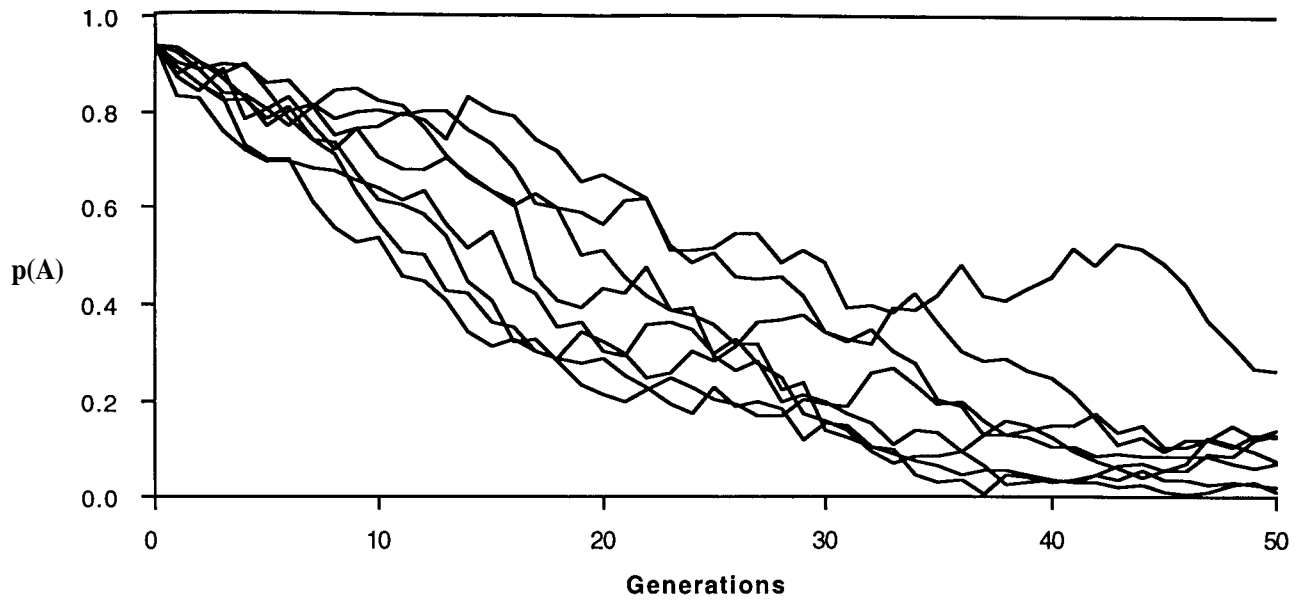
Figures 1-3. The results of eight different simulations of changing allele frequency ( $p(A)$ ) through time (generations) in black stilt. Each simulation assumes a starting allele frequency of 0.94 in black stilt and 0.15 in pied stilt and a population size of 85. Each simulation was replicated eight times for 50 generations. Two parameters were varied: hybrid fitness and rate of gene flow from pied to black stilt.

1A Hybrid fitness 50%; gene flow 15%.

1B Hybrid fitness 50%; gene flow 5%.



2A Hybrid fitness 75%; gene flow 15%.  
 2B Hybrid fitness 75%; gene flow 5%.  
 2C Hybrid fitness 75%; gene flow 3%.



3A Hybrid fitness 90%; gene flow 5%.  
 3B Hybrid fitness 90%; gene flow 3%.  
 3C Hybrid fitness 90%; gene flow 1%.

Table 1. Effect of node type on fecundity of cross

bird 1	bird 2	F1	N eggs	N hatch	N fledge	N >2yr	hatch/ egg	fledge/ egg	2yr/egg	fledge/ hatch	2yr/hatch	2yr/fledge
J x	J	J				39						
		I				12						
		H				6						
		G				3						
total			684	481	242	60	70.3%	35.4%	8.8%	50.3%	12.5%	24.8%
J x	I	J				8						
		I				4						
		H				1						
total			137	102	45	13	74.5%	32.8%	9.5%	44.1%	12.7%	28.9%
J x	H	J				2						
		I				1						
		H				2						
		G				1						
total			67	56	18	6	83.6%	26.9%	9.0%	32.1%	10.7%	33.3%
J x	G	I				2						
		G				1						
total			66	31	7	3	47.0%	10.6%	4.5%	22.6%	9.7%	42.9%
J x	F	I				1						
		H				1						
total			43	38	12	2	88.4%	27.9%	4.7%	31.6%	5.3%	16.7%
J x	D	I				1						
J x	PIED		8									
I x	I		4	4	3							
I x	H		4	4	4							
I x	G		6	6	1							
H	F		4									
H	PIED		2									
G test for plumage effect												
	eggs	hatch	fledge	2yr	total			eggs	hatch	fledge	2yr	total
J x J	684	481	242	60	1467	J x J	4465.1	2970.6	1328.3	245.7	10695.9	
J x I	137	102	45	13	297	J x I	674.0	471.7	171.3	33.3	1691.0	
J x H	67	56	18	6	147	J x H	281.7	225.4	52.0	10.8	733.6	
J x G	66	31	7	3	107	J x G	276.5	106.5	13.6	3.3	500.0	
J x F	43	38	12	2	95	J x F	161.7	138.2	29.8	1.4	432.6	
total	997	708	324	84	2113	total	6884.0	4646.2	1873.0	372.2	16176.8	
						a =	11661.1	G =	18.87	0.1 > P > 0.05		
						b =	13775.4					
						c =	14053.1					
						d =	16176.8					

Table 2. Interactive effect of sex and node type on fecundity of cross

female	male	N eggs	N hatch	N fledge	N >2yr	hatch/ egg	fledge/ egg	fledge/ hatch		
J x	J	684	481	242	60	70.3%	35.4%	50.3%		
J x	I	74	59	29		79.7%	39.2%	49.2%		
I x	J	19	15	8		78.9%	42.1%	53.3%		
J x	H	28	26	12		92.9%	42.9%	46.2%		
H x	J	20	11	4		55.0%	20.0%	36.4%		
J x	G	31	25	7		80.6%	22.6%	28.0%		
G x	J	31	4	0		12.9%	0.0%	0.0%		
J x	F	4	4	2		100.0%	50.0%	50.0%		
F x	J	28	28	10		100.0%	35.7%	35.7%		
I x	G	0	0	0						
G x	I	6	6	1		100.0%	16.7%	16.7%		
I x	H	4	4	4		100.0%	100.0%	100.0%		
H x	I	0	0	0						
I x	I	4	4	3		100.0%	75.0%	75.0%		
G test for male effect										
	eggs	hatch	fledge	total		eggs	hatch	fledge	total	
I male	74	59	29	162	I male	318.5	240.6	97.7	824.2	
H male	28	26	12	66	H male	93.3	84.7	29.8	276.5	
G male	31	25	7	63	G male	106.5	80.5	13.6	261.0	
F male	4	4	2	10	F male	5.5	5.5	1.4	23.0	
total	137	114	50	301	total	674.0	539.9	195.6	1717.8	
					a =	1077.6	G =	2.21	NS	
					b =	1409.6				
					c =	1384.8				
					d =	1717.8				
G test for female effect										
	eggs	hatch	fledge	total		eggs	hatch	fledge	total	
I female	19	15	8	42	I female	55.9	40.6	16.6	157.0	
H female	20	11	4	35	H female	59.9	26.4	5.5	124.4	
G female	31	4	0	35	G female	106.5	5.5	-0.0	124.4	
F female	28	28	10	66	F female	93.3	93.3	23.0	276.5	
total	98	58	22	178	total	449.3	235.5	68.0	922.4	
					a =	526.7	G =	27.63	P < 0.001	
					b =	752.8				
					c =	682.4				
					d =	922.4				

## Appendix 1. Species concepts

- Nominalist (Leibniz & Locke)  
"Nature produces individuals and nothing more ... species have no actual existence in nature. They are mental concepts and nothing more ... species have been invented in order that we may refer to large numbers of individuals collectively" (Bessey 1908).
- Essentialist (Plato)  
"We count as many species as different forms were created in the beginning" (Linnaeus 1736).
- Morphological (phonetic, typological)  
"A species is a group of individuals which produce their like within definite limits of variation, and which are not connected with their nearest allied species by insensible variations" (Wallace 1900)  
  
... when a naturalist can unite two forms together by others having intermediate characters, he treats the one as a variety of the other..." (Darwin 1859).
- Biological (Ray)  
"Species are groups of actually or potentially interbreeding populations which are reproductively isolated from other such groups" (Mayr 1942)
- Evolutionary  
"An evolutionary species is a lineage (an ancestral descendant sequence of populations) evolving separately from others and with its own unitary evolutionary role and tendencies" (Simpson 1961).  
  
"A species is a single lineage of ancestral descendant populations or organisms which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate" (Wiley 1978)
- Ecological  
"A species is a lineage (or a closely related set of lineages) which occupies an adaptive zone minimally different from that of any other lineage in its range and which evolves separately from all lineages outside its range" (Van Valen 1976)
- Phylogenetic  
"A species is the smallest diagnosable cluster of individual organisms within which there is a parental pattern of ancestry and descent" (Cracraft 1983).
- Recognition  
"A species is that most inclusive population of individual biparental organisms which share a common fertilization system" (Paterson 1985).

- Cohesion  
"A species is the most inclusive population of individuals having the potential for phenotypic cohesion through intrinsic cohesion mechanisms" (Templeton 1989).
- Internodal  
"Individual organisms are conspecific in virtue of their common membership of a part of the genealogical network between two permanent splitting events or between a permanent split and an extinction event" (Kornet 1993)
- Genotypic cluster  
"Species are groups that remain recognizable in sympatry because of the morphological and genotypic gaps between them" (Mallet 1995)