The Puerto Rican Parrot

Genetic Assessment of the Captive Breeding Program

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The Puerto Rican parrot *Amazona vittata* is one of the most critically endangered birds in the world. Historically, the Puerto Rican parrot was abundant, probably numbering in the millions throughout the island of Puerto Rico and its satellite islands. The parrots were found in most of the major habitat types on the island; from the moist coastal forest to the montane rainforest in the northeast, to the moist limestone and coastal forests of the northwest, and throughout the forested central Cordillera. The low montane habitats originally covered about 35% of the island and it was probably the major habitat of the parrot.

By the early twentieth century, the Puerto Rican parrot disappeared from all satellite islands, and the mainland population became fragmented. By 1937, only a single isolated population was left, and they were confined to the rainforest of the Luquillo Mountains.[1] Surviving Puerto Rican parrots may still be found only there.

In addition to historical factors that led to the decline of the Puerto Rican parrot, problems continued through the early and mid-1900s.[1] The isolated Luquillo population of Puerto Rican parrots became even more vulnerable to storms and hurricanes because all major weather systems come through the northeast region of the island. This population also became vulnerable to early forest management policies that led to increased accessibility of remote areas. It is not uncommon for Puerto Rican parrots to engage in vicious, even deadly territorial combats over the defense of nest sites, and, during World War II, many trees of the species preferred by the parrots for nests *(Cyrilla racemiflora)* were selectively cut, not only to provide a source of energy in the form of charcoal, but to create jobs. This practice resulted in a scarcity of optimal nest sites. The parrots also contend with natural enemies such as honeybees *(Apis mellifera)* that take over nest cavities, and minor predators such as the Puerto Rican boa *(Epicrates inornatus)* and rats *(Rattus rattus)* that are capable of entering parrot nests and destroying eggs. The major predator of the Puerto Rican parrot appears to be the Red-tailed Hawk *(Buteo jamaicensis)* which probably accounts for the majority of parrot mortality. The average annual mortality rate is high among first year parrots, approximately 32%, and declines to approximately 9% for adult parrots.

Two other significant problems have been with the Warble Fly *(Philornis pici)* that parasitizes parrot nestlings, sometimes causing death, and with an ecological competitor, the Pearly-eyed Thrasher *(Margarops fuscatus)*. The thrashers are also cavity nesters and compete with the parrots for nest sites. Because the thrashers are relatively recent invaders of Puerto Rico, it has been speculated that the parrots have not yet evolved adequate defense mechanisms against the thrashers' aggressive and predacious behavior.

These factors combined, plus the delayed sexual maturity of the Puerto Rican parrot to four or five years of age, have resulted in considerable reproductive failures: 74% to 88% of all nests prior to 1973.[2] The annual rate of population decline became spectacular: it escalated from approximately 8% in the 1950s to over 41% in the mid-1960s, before dropping to 13% by 1971.[1] Population declines finally leveled off during the 1970s, but not without severe consequences. The number of Puerto Rican parrots decreased by two orders of magnitude: from an estimated 2,000 in 1937 to approximately 200 in 1953/54 to a minimum of 13 in 1975.[2] The parrot population has remained relatively stable over the last decade, and much of this stability is due to labor intensive management of the parrot by federal and commonwealth biologists and other dedicated personnel and volunteers.

Conservation of the Puerto Rican Parrot

Management of the wild population of Puerto Rican parrots has included several actions aimed at improving nesting success. Most of these actions involved enhancing natural nest cavities, providing alternative artificial nest cavities for both Puerto Rican parrots and Pearly-eyed Thrashers, and maintenance of nests to prevent water leaks, and honeybee takeovers.[1,2] Also, intense watches (from sun up to sun down, from approximately February to June) of all known active nests are conducted from nearby blinds in the forest.[3] Not only are behavioral observations made on the parrots from the blinds, but interventions are made on behalf of the parrot to safeguard nests from total failure. In some cases, wet or unattended eggs are brought to a nearby aviary for artificial incubation, or sick or uncared-for nestlings are brought in for medical attention. Once hatched or recuperated, nestlings might be returned to their original nest. Sometimes nestlings are exchanged between nests to ensure that otherwise failing nests would fledge at least one or more young. As a result, broods of genetically unrelated nestlings are sometimes mixed in a single nest, and genetically related nestlings fledge from different nests.

After about 14 years (since 1973), nest success increased from a minimum of approximately 11% to approximately 68%,[2] but the number of annual breeding pairs of parrots and the annual population growth rate remained low. Only two to five pairs of Puerto Rican parrots produced an average of 1.6 young per year.[2] Prior to Hurricane Hugo in 1988, there were about 45 to 47 Puerto Rican parrots in the wild: after Hurricane Hugo there were approximately 24. Today the number of Puerto Rican parrots in the wild is still less than 30.

The Captive Breeding Program

The wild population of the Puerto Rican parrot, *Amazona vittata,* has been slowly increasing from a low of

13 individuals in 1975 to about 24 in 1991. As a conservation measure, a captive population was founded by taking 17 eggs or nestlings from the wild between 1973 and 1983.

The primary purpose of the captive breeding program is to release captive-produced parrots into the wild to strengthen the wild population. Between 1979 and 1990, seven pairs of captive Puerto Rican parrots produced 58 offspring, 18 of which fledged from wild nests.[4] There is some evidence that at least one of these parrots, a female, became a successful breeder at a traditional wild nest-site.[1] Also, in 1985, three captive-produced parrots were released into the wild on their own as post-fledglings[1] and one (a male) recently became a successful breeder. It is unknown how many other captive-produced parrots have become members of the wild breeding population.

Although there are currently over 60 Puerto Rican parrots in captivity at the U.S. Fish and Wildlife Service's Luquillo aviary in El Yunque, Puerto Rico, the captive breeding program has not been as productive as that of the closely related but less threatened Hispaniolan parrot, *A. ventralis*.[4] Between 1979 and 1985, only four of the original captive founders (those parrots collected from the wild to establish the captive population) successfully produced offspring. Although four more original founders produced offspring between 1986 and 1990, all of these matings were with offspring of the first four breeding founders. Therefore, the genetic makeup of the captive population is biased towards the first four captive breeding founders.[4] The average number of offspring produced in captivity per year has been 5.3. In addition to the successful breeding pairs, there have been about nine other unsuccessful pairings of captive Puerto Rican parrots; the principal problem has been with egg infertility.

A captive flock of Hispaniolan parrots was also established at the same time as the captive flock of Puerto Rican parrots.[5] The Hispaniolan parrots are used as research surrogates and as foster parents for the Puerto Rican parrots. They incubate Puerto Rican parrot eggs and raise Puerto Rican parrot young. Young Hispaniolan parrots have been fostered into nests of captive Puerto Rican parrots to test the parenting skills of new breeders and have been substituted into wild Puerto Rican parrot nests when the Puerto Rican parrot nestlings needed medical attention at the aviary.

The Hispaniolan parrots are also frequently used for research. Results of experimentation on the Hispaniolan parrots, such as clutch enhancement (double clutching and sequential egg removal), free-flying releases of captive-produced young , and radiotelemetry, have all led to successful applications to the Puerto Rican parrot.[1,6,7] Research on artificial insemination with the Hispaniolan parrot has demonstrated also that some fertility problems among the Puerto Rican parrots might be overcome by using this technique.[8]

Originally, four confiscated Hispaniolan parrots (two males and two females) were obtained from federal law enforcement agents.[1] In 1987, six young Hispaniolan parrots were donated to the captive breeding program. By 1988, nine of these Hispaniolan parrots survived and were the captive founder group. A total of 16 pairs of Hispaniolan parrots have produced offspring over the years, but because six of the nine captive Hispaniolan parrot founders are relatively young birds, most of the captive parrots are descended from the original four birds.[4]

Both captive populations of Puerto Rican parrots and Hispaniolan parrots were founded by only a few individuals. All were maintained on the same diet, housed in the same type of cages, and exposed to the same environmental conditions.

Identical nestboxes and nesting materials have also been provided to both species. When eggs of either species were artificially incubated, they were in identical incubators and under the same temperature and humidity conditions. Also, when young of either species were hand-reared, they were housed in the same environment and fed the same diet. Yet, despite the above similarities, there was a large difference in reproductive success. The Hispaniolan parrots outperformed the Puerto Rican parrots more than two to one in the number of offspring and almost three to one in the number of pairs producing viable offspring.[4]

There has been no intentional inbreeding (the mating of close relatives) among the captive Puerto Rican parrots, but in the wild there has been some evidence of inbreeding.[1] For example, a pair of known siblings fought their parents for a nest site in 1974. The father was lost, the daughter left the area, and the mother and son made a breeding attempt the following year that resulted in a deformed, nonviable chick. Also, in 1986 and 1987, at least one nestling out of every brood produced by one wild breeding pair of Puerto Rican parrots developed normally for the first 21 to 28 days, after which time growth stopped. Pin feathers fell out, skeletal abnormalities became apparent, and secondary infections ensued. None of the afflicted nestlings responded to several forms of medical treatment, nor could an etiological agent be identified by research veterinarians of the U.S. Fish and Wildlife Service, or the University of Florida's College of Veterinary Medicine. All nestlings eventually died. The genetic relationship between the two breeding adults in the last example was unknown.

In the previously described case of a captive-produced female successfully breeding at the wild nest she fledged from, her mate was her foster father.[1] Although the parrots in this last example were genetically unrelated (the female was raised as the foster-offspring in the male's nest), they may be considered behaviorally related. Therefore, questions arose about kin recognition and mate choice in the Puerto Rican parrot and about the amount of genetic variation in the diminished population. Because the numbers of breeding pairs of Puerto Rican parrots in captivity and in the wild remained low, it was suspected that inbreeding may be one of the causal factors.

Inbreeding

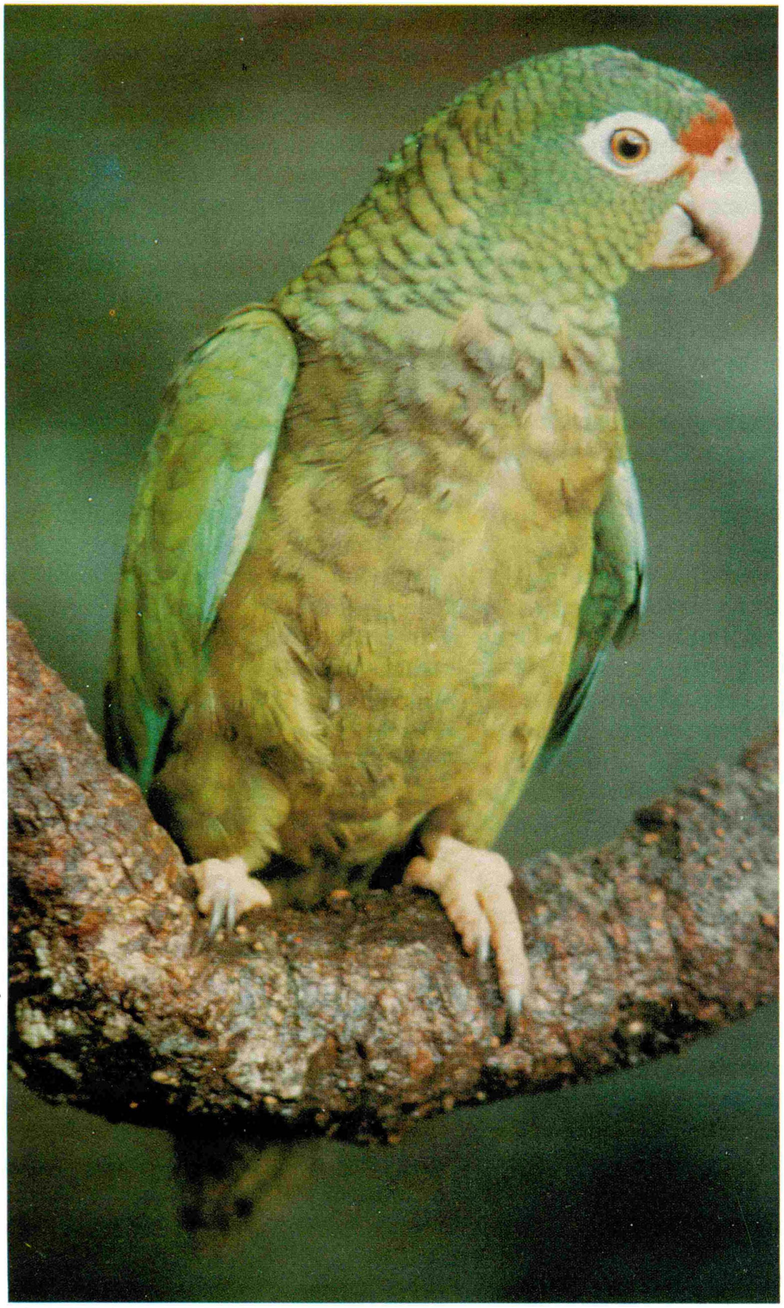
Inbreeding is the mating between close relatives that results in a decrease in genetic variation and an increase in the level of relatedness between individuals in a population. Inbreeding depression is a decline in fitness among inbred individuals, and may be seen after only one generation of inbreeding.[9] For example, inbreeding depression affects characters associated with reproduction such as fecundity, fertility, litter size, developmental rate, viability of offspring, sex ratio of the offspring, age of sexual maturity, behavioral traits related to nest building, and competitive ability.[10]

The few researchers who measured the level of inbreeding in natural bird populations indicated that fewer than 2% of the breeding pairs were parents and offspring, or full siblings.[11] At least 13.6% of breeding wild Puerto Rican parrots (2/16 pairs between 1973 and 1990) were such first degree relatives.[4]

Puerto Rican parrots at the Luquillo Aviary, 1986.

Collecting semen from a captive Hispaniolan parrot, Luquillo Aviary, 1988. Egg fertility was achieved by artificial insemination in Hispaniolan parrots; another potential tool to overcome fertility problems of the captive Puerto Rican parrots.

Photos by U.S. Fish and Wildlife Service personnel, James W. Wiley, Gerald D. Lindsey and M. Kelly Brock (formerly with USFWS)



The Puerto Rican parrot.

Hispaniolan parrot at the Luquillo Aviary, 1984.

It is unknown whether this was a normal level of inbreeding or a consequence of small population size. Regardless, it was one of the highest values reported for a natural population of birds.

In general, there are mechanisms for avoiding or reducing the incidence of inbreeding in natural populations. For example, young may leave their natal areas or adults might return to different breeding areas every year. Also, once a probable relative is recognized, either by location or prior association, some avoidance mechanism may be engaged to prevent inbreeding. Kin recognition seems to be one of the most important mechanisms for avoiding inbreeding, especially when sexually mature close relatives occur in the same group or local area. When kin are recognized they may fail to court, they might reject courtship advances, or fail to come into breeding condition altogether.[12]

Photos by U.S. Fish and Wildlife Service personnel, James W. Wiley, Gerald D. Lindsey and M. Kelly Brock (formerly with USFWS)

Puerto Rican parrot nestlings from wild nests were fitted with radio collars in Luquillo Forest, El Yunque, Puerto Rico, 1987. Movements of the young were tracked for four to seven months during a study to determine home ranges, and to determine when young of the year integrate into the wild flock.

No data are available on kin recognition in the Puerto Rican parrot, but some of their behaviors might be regarded as means of avoiding close inbreeding. For example, the parrots are monogamous and mate for life.[1] Generally, this is an advantage for a small island population of long-lived birds because it eliminates the need to seek a new unrelated mate each year. Furthermore, successfully breeding wild Puerto Rican parrots usually return to the same nesting site year after year[1] which may prolong recognition between parents and older offspring. Young of the year tend to stay with their parents until the start of the next breeding season,[1] and this association may allow older birds (offspring of the same adults from previous years) to recognize their newest siblings. Also, if territorial yet nonbreeding pairs of parrots that are often present in vicinities of active nests[1] are immature parrots, then perhaps pair bonding occurs at an early age. If kin recognition correlates with early associations and wanes after periods of separation,[12] then it is particularly advantageous for the parrots to find unrelated partners as soon as possible. In fact, information from banded Puerto Rican parrots is starting to suggest that some members of new breeding pairs in the wild are relatively young birds.

One factor that may have recently impeded the avoidance of inbreeding in the Puerto Rican parrot is a lower probability of finding an unrelated partner because of drastic population declines and small population size. This could be a plausible explanation for the relatively high incidence of known inbreeding in the wild, and it may have had a detrimental impact on the genetic status of the captive founder group.

The Luquillo Aviary, El Yunque, Puerto Rico, 1986. The building was originally constructed by the Civilian Conservation Corps during the 1940s for use as a hurricane-proof military barracks. The building was renovated for use as an aviary in 1973, and updated in design again in 1987.

DNA Fingerprinting

To determine if inbreeding depression is a potential problem in the captive breeding program for the Puerto Rican parrot, the genetic relationships among the founders were investigated with a technique known as "DNA fingerprinting."[13]

Deoxyribonucleic acid (DNA) is the basic biochemical component of living organisms. DNA, in the form of chromosomes, contains all of the information an organism needs to live and reproduce. Some DNA codes physical characteristics, such as eye color and hair texture, some DNA codes all the proteins and enzymes bodies need to function, and some DNA identifies individuals. It is the latter type of DNA, called "minisatellite DNA,"[13] that we use for DNA profiling.

No two people, nor any two living organisms (with the exception of identical twins and clonal organisms) have the exact same DNA profile; in the same manner, our fingerprints are individualistic and unique. This is, in fact, why the technique is called DNA "fingerprinting".

To obtain this information, DNA is purified from biological tissues, such as hair roots, blood, or skin, and is digested with a restriction enzyme that cuts whole DNA molecules into fragments.[14] These fragments are then separated by size by gel electrophoresis, transferred to a nylon membrane, and probed with a radioactively-labeled piece of minisatellite DNA. The blot is then exposed to x-ray film, which results in a series of black bands that resemble bar codes on grocery packages that identify the product when scanned. DNA fingerprints can be examined in two basic ways. First, because relatives share different proportions of their genetic material, the relationship between two individuals may be estimated by the proportion of shared bands in their DNA fingerprints. Consequently, the degree of relatedness may be estimated for individuals with unknown genetic relationships by comparing the level of bandsharing to the level of bandsharing in the DNA fingerprints of known relatives. Secondly, DNA fingerprint markers may be associated with genes whose variation affect fitness. Therefore, the level of variation in DNA fingerprint may be related to the level of variation of genes that effect fitness. Therefore, DNA fingerprints can be used to measure the degree of genetic relatedness between pairs of individuals in a population and to estimate the relative amount of genetic variation in a population; the two most important genetic components effected by inbreeding.

It was suspected that the captive population of Puerto Rican parrots is more inbred than the captive population of Hispaniolan parrots because the founders of the latter were drawn from a much larger population. It was also suspected that inbreeding depression is limiting the captive breeding program and the overall recovery of the species. The objective, therefore, was to examine the average level of bandsharing from DNA fingerprints of captive Hispaniolan parrots with known genetic relationships and compare them to the average level of bandsharing from DNA fingerprints of the captive founder Puerto Rican parrots. The second objective was to establish whether or not the level of bandsharing between the DNA fingerprints of males and females correlated with breeding success. If so, then DNA fingerprints could be used to identify pairs that may be the most distantly related in the group, which would then increase the probability of producing viable offspring.

The average level of bandsharing in the DNA fingerprints of unrelated Hispaniolan parrots was 19%, 63% among first degree relatives (parents and offspring, or full-siblings), and 39% among second degree relatives (half-siblings, aunts/nephews, uncles/nieces, grandparents/grandchildren). These bandsharing estimates are similar to those reported for other avian species and for humans.

The average level of bandsharing in the DNA fingerprints of the captive founder Puerto Rican parrots was 41%, which was equivalent to second degree Hispaniolan parrot relatives. It was concluded, therefore, that the Puerto Rican parrots are inbred as a result of population attrition and that the captive population was founded by second degree relatives. It was not surprising, then, to find that pairs of males and females with the lowest levels of bandsharing were most successful at producing offspring. For example, bandsharing information was obtained from the DNA fingerprints of 12 of 13 pairs of Puerto Rican parrots, and ten of 12 pairs of Hispaniolan parrots. It was found that the average level of bandsharing for successful breeders was 34% for the Puerto Rican parrots and 29% for the Hispaniolan parrots. Although these values were not statistically different, the average level of bandsharing between unsuccessful Puerto Rican parrots (those who never produced viable offspring) was significantly higher, 47%.

Because of the high level of genetic similarity among the captive Puerto Rican parrots, it was concluded that the low productivity is due, in part, to inbreeding depression. Indeed there was a significant difference in the level of bandsharing between captive pairs that successfully produced offspring and those that did not. Actually, only 38% of captive pairs of Puerto Rican parrots had low levels of bandsharing (less than 40%) whereas all ten captive pairs of unrelated Hispaniolan parrots, of which 90% successfully bred, had low bandsharing coefficients. This was a critical finding for the captive breeding program because it confirmed that the difference in fecundity between the Hispaniolan parrots and the Puerto Rican parrots was primarily biological in nature, and was not due to failures in husbandry techniques.[15]

As mentioned previously, the principal problem for the Puerto Rican parrot in captivity is egg infertility. Although management cannot be ignored, the solution must come from a biological approach, for example, the identification of genetically (as well as behaviorally) compatible pairs using DNA analysis instead of traditional pedigree analysis. Also, by recognizing the biological problem (low productivity), the goals of the program can be altered to conform to the lower reproductive potential of an inbred group rather than the reproductive potential of a genetically diverse group. Fewer resources may then be allocated to factors that probably will not affect an increase in the productivity of the captive Puerto Rican parrot.

In addition to being indicators of the degree of relatedness of a group of animals, bandsharing coefficients also reflect the level of genetic variation at DNA fingerprint loci.[16,17] The large number of alleles per locus and the high level of heterozygosity at DNA fingerprint loci in the Hispaniolan parrots are comparable to that reported for other species.[13,18,19] For example, 17 different alleles were estimated at one DNA fingerprint locus among the nine founder parrots. Furthermore, in a pedigree of 18 Hispaniolan parrots, only one parrot seemed to be homozygous for one of those alleles.

Comparatively, there seem to be substantially fewer alleles and lower levels of heterozygosity at DNA fingerprint loci in the captive founder Puerto Rican parrots. This is also true for some protein coding loci in the

Puerto Rican parrots. The loss of allelic diversity and heterozygosity in the Puerto Rican parrot occurred as a result of several factors, including the attrition of the wild population, the taking of parrots for captivity when the wild population reached its lowest numbers, and from chronically few breeding pairs in both the wild and in captivity.

After one generation of captive breeding, the level of heterozygosity decreased further. However, our analysis indicated that the loss of alleles has slowed, and heterozygosity has been maintained in the captive population by bringing a representative of all new wild breeding pairs into captivity over the years, even though the wild Puerto Rican parrots are also inbred. It has been suggested that genetic variation at loci such as those that comprise DNA fingerprints may correlate with losses at loci that affect fitness. For example, variation at the major histocompatability complex (MHC) is critical to the immunological response of an organism to pathogens. The loss of alleles at these loci could have devastating results for populations of endangered species during an epizootic event. In fact, loss of variation at several loci, including the MHC, in the cheetah has led to a high vulnerability to disease, poor reproduction in captivity and in the wild, and high juvenile mortality.[20]

Although alleles lost thus far from the genome of the Puerto Rican parrot cannot be regained, the remaining variation may be maintained and further losses of alleles prevented if all the parrots contribute to the gene pool. This is particularly important for all surviving original founders and all additional wild parrots added to the captive flock. This is critical to the genetic health of the species because the captive founders, today being the only known survivors of the population when it reached its lowest number, probably contain most of the genetic variation in the species. Hence, the captive population is a vital genetic reservoir for the Puerto Rican parrot. Relatives of the captive founder parrots that remained in the wild may have some variation not present among the captive founders, therefore representation by their descendants in the captive flock is essential. Genetic deterioration in the captive breeding program will continue unless all parrots produce offspring and contribute to the future of the gene pool, and the captive population is increased as rapidly as possible.

The use of DNA fingerprinting to investigate the genetic status of the Puerto Rican parrot has resulted in important findings that make significant contributions to the conservation of this endangered species, and serves as a model for others. Because there was an association between reproductive success and the levels of bandsharing in DNA fingerprints, DNA profiling was used (in conjunction with behavioral compatibility criteria) to identify pairs of males and females with low levels of bandsharing, thus increasing the likelihood that they will produce viable offspring.

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