Italian Journal of Zoology

ISSN: 1125-0003 (Print) 1748-5851 (Online) Journal homepage: https://www.tandfonline.com/loi/tizo20

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To cite this article: Renato Massa , Maurizio Sara , Matteo Piazza , Cornelia Di Gaetano , Margherita Randazzo & Goffredo Cognetti (2000) A molecular approach to the taxonomy and biogeography of African parrots, Italian Journal of Zoology, 67:3, 313-317, DOI: 10.1080/11250000009356330

To link to this article: https://doi.org/10.1080/11250000009356330

Ital. J. Zool., 67: 313-317 (2000)

A molecular approach to the taxonomy and biogeography of African parrots

RENATO MASSA
Dipartimento di Scienze dell'Ambiente e del Territorio,
Università degli Studi di Milano Bicocca,
piazza della Scienza 1, I-20126 (Milano)

MAURIZIO SARÀ
MATTEO PIAZZA
CORNELIA DI GAETANO
MARGHERITA RANDAZZO
GOFFREDO COGNETTI
Dipartimento di Biologia animale, Università degli Studi di Palermo,
via Archirafi 18, I-90100 (Palermo)

ABSTRACT

Random amplified polymorphic DNA analysis of blood samples from eight African parrot species was performed to study the genetic relationship within the genus *Poicephalus* and among *Poicephalus* and the two other main African parrot genuses, *Agapornis* and *Psittacus*. To this end, DNA from six *Poicephalus* species, one species of the *Agapornis* group and the single *Psittacus* species was analysed. The amplification pattern was then converted into a binary matrix and scored by the unweighted pair-group method algorithm. The resulting dendrogram showed a neat separation of all the *Poicephalus* on one side, from *Psittacus-Agapornis* on the other side. Among the six analysed species of *Poicephalus*, two larger clusters occurred, one containing four species belonging to the *P. meyeri* superspecies and the other one containing the two species pertaining to the *P. robustus* superspecies. The larger one of these is further subdivided in two smaller clusters, each one containing two species, that is *P. meyeri-P. cryptoxanthus* and *P. senegalus-P. rufiventris*, respectively. These results are consistent with the hypothesis that the genus *Poicephalus* is a natural assemblage that, after undergoing a very early separation from *Psittacus* and *Agapornis*, was subsequently affected by a number of discrete speciation events, especially during episodes of aridity resulting in fragmentation of forest habitats.

KEY WORDS: *Poicephalus* - DNA - speciation.

ACKNOWLEDGEMENTS

This work is dedicated to the memory of Prof. Goffredo Cognetti. It was made possible by the intra-mural grant provided to this end by the Rector of the University of Milano, Prof. Paolo Mantegazza. An earlier version of the manuscript was read by Prof. Mike Perrin who gave useful suggestions to improve it. Dr. Lorenzo Crosta kindly helped to take some blood samples. All this help is gratefully acknowledged.

(Received 22 February 2000 - Accepted 14 June 2000)

INTRODUCTION

Continental African parrots are currently classified in four genera: *Poicephalus*, with nine species (eleven according to (Solms L. *et al.*, 2000, *Abstract* in South Afr. Soc. Congr. 2000), *Agapornis*, with eight, and *Psittacus* and *Psittacula*, with one species each. *Psittacus* and *Poicephalus* are continental African endemics, *Poicephalus* being the most diverse and most widely distributed African parrot genus. *Agapornis* includes eight continental endemic species and a ninth one, *A. cana*, endemic to Madagascar. *Psittacula* has no species endemic to continental Africa, the genus being mainly found in Asia and the Mascarene archipelago where it is represented by 13 living species, just one of which, *P. krameri*, extends its range to central and north eastern Africa.

The larger two former species of *Poicephalus*, the red-fronted parrot *P. gulielmi* and the cape parrot *P. robustus*, are usually considered as forming a superspecies (*P. robustus* supersp.) while the remaining seven are considered as members of another superspecies (*P. meyeri* supersp.; Forshaw, 1978; Fry *et al.*, 1988). The *P. robustus* superspecies members and the yellow-faced parrot *P. flavifrons* are mostly forest-dwelling birds, while the six other smaller species (*P. senegalus, P. rufiventris, P. crassus, P. cryptoxanthus, P. meyeri, P. rueppellii)* are birds of drier habitats such as baobab savannah and riverine forests. Their geographical distribution is generally allopatric or parapatric for the *P. meyeri* superspecies while it is allopatric and highly fragmented for the *P. robustus* superspecies, suggesting that speciation within this genus may have occurred recently, perhaps as a consequence of recent ecological disruption with dramatic habitat changes in the latter half of Pleistocene.

In order to collect preliminary information on the genetic relationships within the genus *Poicephalus* and between *Poicephalus* and the two other main African parrot genera, *Agapornis* and *Psittacus*, we performed a random amplified polymorphic DNA (RAPD) analysis of the DNA of eight African parrot species including six *Poicephalus*, one species of the *Agapornis* group, and the single *Psittacus* species.

MATERIALS AND METHODS

Blood samples were obtained from the wing vein of 22 individual African parrots pertaining to eight different taxa (*Poicephalus robustus suahelicus, P. gulielmi massaicus, P. senegalus mesotypus, P. rufiventris, P. meyeri matschiei, P. c. cryptoxanthus, Psittacus e. erithacus, Agapornis roseicollis*), all of them kept in captivity at the Parrot Breeding and Research Centre of the University of Milano. Other important taxa of the genus *Poicephalus*, such as *P. rueppellii, P. crassus, P. flavifrons, P. r. robustus, P. r. fuscicollis* could not be taken into consideration for the present study as no specimen was available to us. All samples were stored at -80° C until analysed for their genomic DNA.

DNA preparation

Genomic DNA was extracted from blood cells adapting the classical method of Herrmann & Frischauf (1987) which involves

suspension in sodium laurysulfate/proteinase K, followed by phenol and chloroform extractions and isopropanol precipitation. RNA was eliminated by RNAse treatment.

RAPD-PCR

Random primers were purchased from Operon Technologies (Alameda, Ca.). Several primers were tested and five of them gave a pattern in all the specimens. Amplification conditions were those described by Williams *et al.* (1990) with the only difference being that we employed the 'Stoffel fragment' of Taq polymerase (Perkin-Elmer, Ca.). Compared with normal Taq polymerase, the Stoffel fragment is reported to be more thermostable, allowing higher denaturation temperatures (Lawyer *et al.*, 1993). This results in electrophoretic patterns at a very good degree of reproducibility. Since the purity of water is critical in this procedure, all water solutions were made in Milli Q-plus (Millipore) filtered water, and two blanks containing water instead of DNA were also tested in each amplification.

Electrophoresis

Small aliquots from the amplification products were run on 2% agarose gels in the presence of ethidium bromide. We used as a marker the 100-bp ladder produced by Pharmacia (Uppsala). Amplification patterns were detected on a 312-nm UV screen and photographed. Enlarged prints were used for further calculations.

Computer analysis

The five primers screened in the 22 individual specimens amplified a complex pattern of polymorphic genomic DNA (Appendix I). Each band in each specimen was scored as 1 if present (marker) or 0 if absent ('null allele'), according to Lynch & Milligan (1994). Letting M and m denote the marker and the null allele at a locus, and p and q their frequencies, the only observable quantity for a locus in a RAPD profile is the fraction of individuals in the population with (1 - x) or without (x) the marker. Since the null allele frequency is $q = x^{1/2}$ the resulting matrix (22 specimens × 82 bands, Appendix I) of marker frequencies from single individuals will correspond to the gene frequency matrix. The original matrix was then converted into a distance one (Appendix II), employing the Nei (1972) formula. The distance matrix was treated by the unweighted pair-group method algorithm (UPGMA) in order to cluster the 22 specimens. All these analyses were performed by the NTSYS-PC software (Rohlf, 1993). According to Lynch & Milligan (1993):

$$q = x^{1/2} \cdot [1 - Var(x)] - [1 / 8x^2]$$

where $Var(x) = x \cdot (1 - x)/3$ is the sampling variance of the frequency of null alleles. The Nei (1972) genetic distances among the eight species of African parrots were also clustered by UPGMA.

RESULTS

PCR-amplification of parrot DNA by RAPD fingerprinting yielded a series of discrete fragments. A total of 82 fragments were amplified by the five primers used in the study (Appendix I). Each primer gave a different RAPD profile amplifying from 9 to 22 DNA fragments.

For each primer, several bands are common to all the samples examined. Although co-migration does not necessarily imply identity of DNA fragments (see Discussion below), the data, nevertheless, indicate an overall similarity of the species examined. In particular, primer B15, *Agapornis*, was discriminated by the presence of five bands and by the absence of four bands (Appendix I). The presence of bands that are common to all the samples of a genus but not to others may be diagnostic of the genera, as suggested by Hadrys *et al.* (1992).

The dendrogram in Fig. 1 shows a clear separation of all the *Poicephalus* from the *Psittacus-Agapornis* group on the other side. Among the six taxa of *Poicephalus* analysed, two larger clusters occur, one containing four taxa and another two taxa. The former and larger one of these is further subdivided in two smaller clusters, each one containing two taxa (*P. rufiventris-P. senegalus* and *P. meyeri-P. cryptoxanthus*, respectively).

DISCUSSION

Since the RAPD method does not allow to directly check whether or not electrophoretic bands represent phylogenetically homologous DNA sequences, we cannot state with absolute certainty that bands running in the same way (and therefore showing an apparently identical molecular weight) really represent common sequences of different taxa. Due to this, that is intrinsic to the RAPD method, we cannot express the genetic divergence of African parrots in terms of mutational distance. However, since an identical band migration suggests an identical molecular weight and this, in turn, suggests a very similar DNA sequence, the results of this study suggest that the genus *Poicephalus* is a natural assemblage that underwent an early separation from *Psittacus* and *Agapornis*. In addition, the superspecies *P. meyeri* and *P. robustus*, which are generally accepted by taxonomists, also appear as natural assemblages within the genus *Poicephalus*. Our results also suggest that, within the superspecies *P. meyeri*, two clear-cut

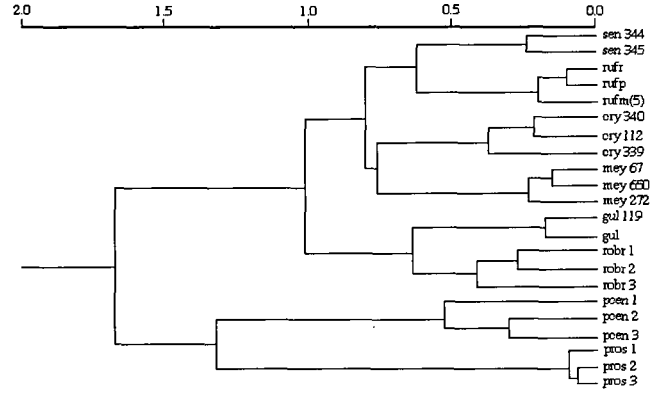

Fig. 1 - Dendrogram showing the Nei's genetic distance among the eight species of African parrots as resulting from UPGMA clustering (see text for further explanation). SEN = *P. senegalus mesotypus*; RUF = *P. rufiventris*; CRY = *P. c. cryptoxanthus*; MEY = *P. meyeri matschiei*, GUL = *P. gulielmi massaicus*; ROB = *P. robustus suahelicus*; PCEN = *Ps. e. erithacus*; PROS = *A. roseicollis*. Letters or numbers following the above characters refer to the single individuals analysed.

sub-clusters occur, the first one including Senegal parrot and orange-bellied parrot, the second one brown-headed parrot and Meyer parrot. Although the conclusions of the present work are preliminar and require confirmation by DNA sequencing techniques, we believe that they are useful and that the RAPD method should deserve attention in the preliminary phases of other phylogenetic studies.

One of the outstanding contributions of Moreau (1966) was in recognising that the ranges of many species in tropical Africa were greatly influenced by climatic changes during the Quaternary. The present available evidence (Diamond & Hamilton, 1980) indicates that temperature fluctuations in tropical Africa during the Quaternary were approximately synchronous with those in other parts of the world and that glacial periods were dry and interglacials were wet. The increased aridity during each glacial period restricted tropical forests to the most climatically stable 'refuge' areas; conversely, in each interglacial, precipitation increased and forests expanded (Diamond & Hamilton, 1980). It has been proposed that such climate dynamics may explain not only forest bird but also forest mammal distribution in Africa (see, for instance, Lawes, 1990, for the distribution of the samango monkey in southern Africa).

The present distribution map of *P. robustus* superspecies shows highly fragmented ranges (see Fry *et al.*, 1988). If one assumes that the formerly continuous range has become fragmented, it may be hypothesized that this process was caused by environmental changes during glacial periods. Each of the populations of both taxa (*P. robustus* and *P. gulielmi*) are now confined to the various forest fragments and may have become adapted to slightly different kinds of habitats (e.g. mangrove forest for *P. r. fuscicollis,* open woodland for *P. r. suahelicus, Podocarpus* 'yellowwooods' for *P. r. robustus*; Forshaw, 1978; Fry *et al.*, 1988). This adaptation in geographic and ecological isolation proceeded so much in the case of *P. robustus* as to give origin to three very distinct taxa that are now believed to deserve the status of full species (Solms *et al.*, 2000). Even during wet periods, when tropical forests expanded, these taxa may have been unable to recolonize new forest patches. At present, the only larger and more or less continuous distribution pattern is shown by *P. r. suahelicus* in the East African open woodland, which corresponds to an adaptation of this taxon to a more open and more widespread habitat. In summary, the present distribution of *P. robustus* superspecies, with a number of highly distinct populations in different forest patches, may reflect a past history of fragmentation and subsequent inability to recolonize new forest habitats after aridity episodes.

Within the *P. meyeri* superspecies, the distinct clustering of *P. senegalus* with *P. rufiventris,* and *P. meyeri* with *P. cryptoxanthus* suggests the possibility of the past existence of two ancient groups, one that we may call the 'orange-bellied' group that may have inhabited the dry sahelian belt from Senegal on the west to the Red Sea on the east, and another that we may call the 'grey-green' group, possibly adapted to more dense riverine forest and widespread south of the Equator, in open woodland and savannah habitats. During some of the glaciation 'arid' episodes, both ranges may have become fragmented, resulting in speciation processes that may have separated *P. senegalus* from *P. rufiventris* north of the Equator and *P. meyeri* from *P. cryptoxanthus* south of it. During a subsequent interglacial period, *P. meyeri* may have expanded its range towards the north, occupying the central part of the sahelian belt that, in the interim, had been vacated by the 'orange-bellied' group, and reaching its present wide geographical distribution in the continent.

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APPENDIX I - *Matrix of marker frequencies from single individuals corresponding to the gene frequency matrix.*

Primer	*P. sen.*	*P. sen.*	*P. cry.*	*P. cry.*	*P. mey.*	*P. mey.*	*P. mey.*	*P. cry.*	*P. guli.*	*P. guli.*	*P. rufi.*	*P. rufi.*	*P. rufi.*	*P. rob.*	*P. rob.*	*P. rob.*	*Psi. eri.*	*Psi. eri.*	*Psi. eri.*	*Agap. ros.*	*Agap. ros.*	*Agap. ros.*
A10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
	0	1	0	1	0	0	0	0	0	1	1	1	1	1	0	1	0	1	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
	1	1	0	1	0	0	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0	0
	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
	1	1	1	1	0	0	0	1	0	0	1	1	1	1	1	1	1	1	0	0	0	0
	1	1	0	0	0	0	0	0	1	0	1	1	1	0	0	0	1	1	0	0	0	0
	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0
	1	1	0	1	0	0	0	0	1	1	1	1	1	0	0	1	0	0	0	0	0	0
	0	1	0	1	0	0	0	0	1	1	0	0	1	1	1	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	1	0	1	0	1	1	1
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	0	0
	1	1	1	1	0	0	1	1	0	1	1	1	1	1	1	1	1	0	1	0	0	0
	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	1
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
	0	0	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1
B14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
	1	1	1	1	1	1	1	1	0	0	1	1	1	0	0	0	0	0	0	1	1	1
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0	0	0	0
	1	1	0	0	1	1	1	0	0	0	1	1	1	0	0	0	1	0	0	0	0	0
	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	1	1	1
	0	0	0	1	0	0	0	0	1	1	0	0	0	1	1	1	1	1	0	0	0	0
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	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
	0	0	0	0	0	1	1	1	1	1	1	1	0	1	0	0	1	0	0	1	1	1
	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
B15	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
F3	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	1	1	1	1	0	0	0
	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
	0	0	1	1	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0
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	0	0	0	0	1	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
F6	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	1	1	1	0	0	0
	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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	0	0	0	0	0	1	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1

APPENDIX II - *Distance matrix obtained from the original matrix of marker frequencies by employing the Nei (1972) formula.*

	P. sen.	*P. sen.*	*P. cry.*	*P. cry.*	*P. mey.*	*P. mey.*	*P. mey.*	*P. cry.*	*P. guli.*	*P. guli.*	*P. rufi*	*P. rufi*	*P. rufi*	*P. robr.*	*P. robr.*	*P. robr.*	*Psi. eri.*	*Psi. eri.*	*Psi. eri.*	*Agap. ros.*	*Agap. ros.*
P. sen	0.17																				
P. cry.	0.48	0.48																			
P. cry.	0.43	0.32	0.22																		
P. mey.	0.48	0.39	1012	1006																	
P. mey.	0.59	0.5	0.66	0.68	0.11																
P. mey.	0.54	0.48	0.39	0.54	0.17	0.16															
P. cry.	0.63	0.64	0.15	0.3	0.47	0.4	0.21														
P. guli.	1629	1099	1035	0.54	1546	1190	0.66	0.54													
P. guli.	1341	0.66	1258	0.39	1546	1190	0.66	0.64	0.13												
P. rufi	0.4	0.38	0.69	0.49	0.58	0.44	0.51	0.56	0.58	0.44											
P. rufi	0.44	0.42	1055	0.53	0.63	0.49	0.48	0.6	0.62	0.48	0.07										
P. rufi	0.48	0.46	1199	0.58	1017	0.61	0.68	0.66	1263	0.68	0.14	0.15									
P.rob.	1394	0.69	1087	0.5	2004	1242	1151	0.67	0.44	0.26	0.42	0.4	0.43								
P.rob.	1059	0.62	1199	0.5	1199	1354	1222	1011	0.62	0.44	0.4	0.44	0.48	0.28							
P.rob.	1027	0.5	1167	0.48	1454	1609	1190	1161	0.59	0.35	0.52	0.49	0.53	0.2	0.3						
Psi. eri.	1357	1183	2189	1491	2189	1939	1519	1714	1337	1183	0.61	0.65	0.69	0.54	0.53	0.67					
Psi. eri.	1506	1109	2808	1417	1422	1354	1445	1927	1263	1109	0.62	1060	1139	0.63	0.55	0.61	0.28				
Psi. eri.	2062	1819	2671	1790	1573	1440	1308	1790	1308	1126	1269	1511	1695	1178	0.63	1440	0.47	0.21			
Agap.ros.	2199	1956	2808	3026	1710	1577	1668	2333	2361	2361	1407	1466	1427	1721	1609	1577	1214	1273	1472		
Agap.ros.	2112	1869	2721	2939	1623	1490	1581	2246	2967	2967	1502	1561	1522	1922	1810	1778	1260	1186	1385	0.087	
Agap.ros.	2135	1892	2051	2962	1646	1513	1381	1864	2298	2991	1525	1584	1546	1945	1833	1801	1283	1363	1631	0.08	0.072