The Auk 115(2):455–464, 1998

EVOLUTION OF NEST-BUILDING BEHAVIOR IN *AGAPORNIS* PARROTS

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ABSTRACT.—Five species in the African lovebird genus *Agapornis* are the only parrots, other than Monk Parakeets (*Myiopsitta monachus*), that construct nests. Four species (*A. personata, A. fischeri, A. lilianae,* and *A. nigrigenis*) build domed nests within cavities, and a fifth (*A. roseicollis*) builds a cup-shaped nest within a cavity. The other members of the genus have nesting behavior that is more typical of other parrots: *A. cana* and *A. taranta* nest in cavities that are lined with nesting material, and *A. pullaria* excavates burrows in arboreal ant or termite nests. To reconstruct the evolution of nest-building behavior in *Agapornis,* I sequenced a 622-bp portion of the cytochrome-*b* gene (mtDNA) and used the sequence data to build a phylogenetic tree. The phylogeny shows that the divergence between the nest-building species and *cana, taranta,* and *pullaria* occurred early in the evolution of the genus. The nest builders form a monophyletic clade, and the small amount of sequence divergence between *personata, fischeri, lilianae,* and *nigrigenis* indicates that they probably should be considered subspecies of a single species. A reconstruction of the evolution of nest-building behavior on the phylogeny indicates that the construction of a domed nest is derived from the habit of lining the nest, because the nesting material is used to build progressively more complex nest structures. Within *Agapornis,* nest building is associated with colonial breeding. The construction of a nest within a cavity may allow breeding pairs to modify and use cavities that otherwise might be unsuitable. This would, in turn, give pairs added flexibility in nest-site choice, thereby facilitating colonial breeding. *Received 5 May 1997, accepted 11 November 1997.*

CAVITY NESTING has evolved multiple times among birds (Collias and Collias 1984). Because cavity nests are safe and well protected, elaborate nest-building behavior is not predicted to evolve in cavity-nesting lineages (Collias and Collias 1984). Parrots present some exceptions to this pattern: tree hollows probably are the primitive nest type of parrots (Eberhard 1997), yet two genera construct complex nests. Monk Parakeets (*Myiopsitta monachus*), which are native to South America, build domed stick nests, and some members of the African genus *Agapornis* construct domed nests within cavities (Forshaw 1989). A survey of the nesting behavior of extant parrots suggests two hypotheses for the evolution of nest-building behavior in the Psittaciformes: (1) nest building evolved from the habit of lining the cavity with nest material; and (2) nest building evolved via nest adoption.

The genus *Agapornis* is an interesting group for studying the evolution of nest-building behavior in parrots because its nine species include examples of most types of nesting behavior observed across the parrot family. Members of the genus *Agapornis* are small, short-tailed parrots that are native to African forests and savannas. Two species (*A. cana* and *A. taranta*) nest in tree holes, and one (*A. pullaria*) uses cavities excavated in arboreal ant or termite nests (Forshaw 1989). In all three of these species, the nest cavity is lined with material (e.g. seed husks, small pieces of bark, grass or leaves) that the female carries tucked in her body feathers (Forshaw 1989). The nesting habits of a fourth species, *A. swinderniana,* are poorly known, but it may nest in termitaria as well (Forshaw 1989). *Agapornis roseicollis* females carry nesting material (strips of bark, leaves, or grass) tucked in their rump feathers and build cup-shaped nests within cavities. Females of the remaining four species (*A. fischeri, A. personata, A. lilianae,* and *A. nigrigenis*) carry nesting material (long stalks and strips of bark) in their beaks and build bulky, domed nests within cavities (Forshaw 1989). The nesting material is woven together, and the resulting structure retains its shape even if removed from the cavity (Dilger 1960, Vriends 1978).

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TABLE 1. Taxonomic characters used by Moreau (1948: table 5) in classifying species in the genus *Agapornis*. Characters in italics are shared characters.

Character	Group A[a]	*A. roseicollis*	Group B[b]
Natal down	White	*Red*	*Red*
Distinct juvenal plumage	*Present*	*Present*	Absent
White skin around eye	*Absent*	*Absent*	Present
Sexual dimorphism	Present	*Absent*	*Absent*
Black bar on central tail feathers	Present	*Absent*	*Absent*
Method of carrying nest material	*In feathers*	*In feathers*	In beak
Form of nest	Pad	Cup	Dome

[a] *A. cana, A. pullaria, A. taranta.*
[b] *A. fischeri, A. personata, A. lilianae, A. nigrigenis.*

The first comprehensive attempt to understand the evolution of the genus *Agapornis* was made by Moreau (1948), who summarized what was known about the distribution, ecology, morphology, and behavior of lovebirds. Using all of this information, he proposed a classification of the genus. Moreau's (1948) grouping of *Agapornis* is based on seven morphological and behavioral characters (see Table 1). The designation of some as "primitive" and others as "advanced" results from the selection of *Loriculus* as the most closely related genus, because members of the latter genus also carry nesting material in their feathers and are sexually dimorphic. *A. roseicollis* appears to be intermediate with respect to the "primitive" (Group A) and "advanced" (Group B) species, because it shares three characters with each group. Placing it as a phylogenetic intermediate would indicate that nest-construction behavior evolved from the habit of lining the nest, reflecting a gradual elaboration of nest building. However, Moreau (1948:236) suggests that *A. roseicollis* and the Group B species are different lineages: ". . . it is curious that of all the Group B birds, *A. nigrigenis*, the member that is geographically nearest to *A. roseicollis*, should be most unlike it. This suggests that, although *A. roseicollis* is intermediate in characters, Group B evolved independently."

Most of our knowledge of the behavior of lovebirds is a result of detailed observations of captive individuals made by Dilger (1960, 1961). His studies included all of the species in *Agapornis* except for *A. swinderniana*, and they provide descriptions of breeding and social behavior. Based on his behavioral observations, Dilger (1960, 1961) characterized *cana, taranta,* and *pullaria* as "primitive," and *fischeri, personata, lilianae,* and *nigrigenis* as the most "highly evolved." The behavior of the remaining species, *roseicollis,* appeared intermediate, leading Dilger to conclude that it arose from the "primitive" species and is ancestral to the "highly evolved" species (Dilger 1960, 1961). Dilger concurred with Neunzig (1926) and Hampe (1957) that *fischeri, personata, lilianae,* and *nigrigenis* probably are subspecies of one species. He also suggested that *taranta* and *pullaria* are more closely related to each other than to other species in the genus, and that they are the closest relatives of *cana,* which is endemic to Madagascar. Although he generally agreed with Moreau's arrangement, Dilger (1960:650) suggested that Group B species are most closely related to *A. roseicollis* and probably were "derived from a *roseicollis*-like ancestor."

In order to determine the relationships among *Agapornis* species, and to reconstruct the evolutionary history of nest building in the genus to test the nest-lining hypothesis, I used mtDNA sequence data to infer a phylogeny for the group. The choice of mtDNA data for phylogeny reconstruction was made for two main reasons: (1) morphological data have proven to be of limited taxonomic value in parrots (Smith 1975, Forshaw 1989); and (2) some of the characters that have been used to define the "primitive" and "advanced" species of *Agapornis* (Moreau 1948; Dilger 1960, 1961) are associated with nest building and would introduce an inappropriate bias (de Queiroz 1996) to this test of the nest-lining hypothesis.

METHODS

Blood feathers were used as sources of DNA for most polymerase chain reactions (PCR) and sequencing reactions; DNA from one of the *A. personata* samples was extracted from buffered blood. Feather sam-

TABLE 2. Feather samples used as sources of DNA for PCR and sequencing.

Source	Band or ID no.
Agapornis cana	
J. Landvater	17L-R-96-53-ALBS
J. Landvater	17L-R-96-54-ALBS
A. taranta	
San Diego Zoo	391108-7EWR-3
San Diego Zoo	495050-SDZ15
A. pullaria	
San Diego Zoo	393091-1GT-846
San Diego Zoo	393105-817
A. roseicollis	
San Francisco Zoo	197-288912
Dickerson Park Zoo	—
A. fischeri	
Dallas Zoo	DZST 053
Dickerson Park Zoo	3608
A. personata	
Fort Wayne Zoo	—
Dickerson Park Zoo	3507
D. Emlen/K. Bright	—
A. nigrigenis	
San Diego Zoo	391505-0127
San Diego Zoo	391513-002
A. lilianae	
Brookfield Zoo	24152
Brookfield Zoo	25100
Loriculus galgulus	
San Diego Zoo	CEM 50
San Diego Zoo	CEM 51

ples from eight of the nine species of *Agapornis* and one species of *Loriculus* (the Blue-crowned Hanging-Parrot [*Loriculus galgulus*]) were taken from captive birds in zoos in the United States and, in one case, from an aviculturist's collection. The *A. personata* blood sample was taken from a pet bird. A list of samples used in this study is provided in Table 2. DNA sequences were obtained from at least two individuals of each species in order to check for intraspecific sequence variation. Within-species variation might be expected in the white eye ring taxa, because they are known to hybridize in captivity (Moreau 1948, Vriends 1978). In most cases, these individuals were unrelated, and where possible from different zoos; the only exception was *A. cana*, for which the samples were from full sibs (and therefore expected to be identical due to the maternal inheritance of mtDNA). The feather samples from each individual were stored in separate bags or envelopes at room temperature. All extractions were done within two months of sample collection. I was unable to locate any zoos or aviculturists in the United States, Europe, or Africa who keep *A. swinderniana*. Because the extraction of ancient DNA from museum skins and the designing of primers necessary for amplifying such DNA were beyond the scope of this study, *A. swinderniana* was not included in the analyses.

DNA extraction from blood feathers was done according to a modified hair lysis buffer extraction protocol (P. Wade pers. comm.). A small (<2 mg) piece of the feather tip was immersed in 400 μL of a hair lysis buffer (0.9% Tween 20, 10 mM Tris pH 8.0, 50 μg/mL Proteinase K, 35 mM DTT) and incubated overnight at 56°C. Following the addition of 500 μL of 5% Chelex, the samples were incubated at 95°C for 30 min, cooled to room temperature, and then microfuged for one minute at maximum speed. Finally, 350 μL of the supernatant were transferred to a new tube and stored at −20°C. The supernatant was used in amplification reactions without dilution or further purification. For all sets of extractions (usually three to six samples each), two negative extraction controls also were performed. Extraction of DNA from the single buffered blood sample was done by first incubating the sample with SSC, TNE, and Protease K, followed by a standard phenol/chloroform extraction and dialysis.

PCR amplification (Saiki et al. 1988) of a portion of the cytochrome-*b* gene (mtDNA) was done using primers L14841 (Kocher et al. 1989) and CB3-H (Palumbi et al. 1991). Amplifications were done using 1 μL of extraction supernatant as template in 10 μL total-volume PCRs that contained a buffer (10 mM Tris/HCl, 50 mM KCl, 2.5 mM $MgCl_2$, pH 8.3), each dNTP at 0.2 mM, each primer at 0.2 μM, and 0.5 units of *Thermus aquaticus* polymerase. The first cycle of the PCR reaction consisted of denaturation for 3 min at 94°C, annealing for 2 min at 50°C, and extension for 3 min at 72°C. This was followed by 35 cycles of denaturation for 30 s at 94°C, annealing for 1 min at 50°C, and extension for 1.5 min at 72°C. The reaction was completed with a single cycle of denaturation for 30 s at 94°C, annealing for 1 min at 50°C, and extension for 4 min at 72°C. With each set of PCR reactions, a negative control was included to permit the detection of any contamination of reagents. Electrophoresis of PCR products in a 0.4% agarose mini-gel (TAE buffer), followed by staining in ethidium bromide (EtBr), was done to check for amplification. The PCR product was then diluted (up to a 1:10 dilution depending on the concentration of product as estimated by the EtBr staining) and used as template for sequencing reactions.

Sequencing of most samples was done using the *fmol* cycle sequencing kit (Promega), using end-labeled ^{32}P, according to the manufacturer's instructions. Both strands of the fragment between L14841 and H15149 were sequenced for an *A. personata* sample and found to match without any discrepancies. For all of the other samples, the 622-bp fragment was

sequenced in two sections: approximately half of the region was sequenced using primer H15149 (Kocher et al. 1989), and the other half with primer CB3-H. The *A. cana* samples were sequenced by the automatic-sequencing facility at Princeton University. Sequences were easily aligned by eye to each other, and to the Night Parrot (*Geopsittacus occidentalis*) sequence published in Leeton et al. (1994); no insertions or deletions were found.

Maximum-parsimony tree searches were done using test version 4.0.0d59 of PAUP* (provided by D. L. Swofford). Maximum-parsimony trees were found using the exhaustive search option, and bootstrap and jackknife resampling with random branch addition were implemented on all trees (2,000 replicates) to assess the resolving power of the data. Trees were rooted with both the *Loriculus galgulus* and the *Geopsittacus occidentalis* sequences (Leeton et al. 1994). *Loriculus* is thought to be a sister genus to *Agapornis* (Dilger 1960); *Geopsittacus* is Australian and a member of a different tribe (Forshaw 1989) and thus is more distantly related. I conducted maximum-parsimony searches with equal weighting of all base positions, and also with codon weights of 3:10:1 (1st: 2nd: 3rd position weights, reflecting the relative number of variable sites at each position). These weightings were calculated from the numbers of base changes occurring at each codon position in the data set. Tree statistics (treelength, and consistency and retention indices) for parsimony trees were calculated using MacClade (Maddison and Maddison 1992), with uninformative characters excluded. Because of the difficulties with calculating treelength for trees that contain polytomies of uncertain resolution ("soft" polytomies), as did the trees in this study, it should be noted that the treelengths are not exact and are likely to be underestimates (Maddison and Maddison 1992). PAUP* also was used to compare alternative tree topologies by performing Kishino-Hasegawa tests (Kishino and Hasegawa 1989).

Maximum-likelihood trees were reconstructed using the quartet-puzzling method (Strimmer and von Haeseler 1996) implemented in the PUZZLE program (Strimmer and von Haeseler 1997), with 1,000 puzzling steps. I used the Hasegawa-Kishino-Yano (1985; HKY) substitution model with the transition/transversion parameter and nucleotide frequencies estimated from the data (exact parameter estimation option). Trees were found assuming uniform substitution rates and Gamma distributed rates. The reliability values generated by PUZZLE reflect the number of times a given group is reconstructed during puzzling steps and are highly correlated with bootstrap values (Strimmer and von Haeseler 1996).

I calculated pairwise distances among sequences using: (1) the uncorrected percentage sequence divergence, with all positions given equal weight and no substitution model assumed; and (2) the maximum-likelihood distance, which was found using program DNAML in PHYLIP 3.5 (Felsenstein 1993). The latter distances were calculated to estimate divergence times using the crane calibration of Krajewski and King (1996); a rate calibration is not available for parrots. To test whether the *Agapornis* data were consistent with a molecular clock (i.e. whether use of the above calibration was valid), a maximum-likelihood tree search was done using PAUP* (equally weighted data, HKY substitution model, transition/transversion ratio = 2), with and without enforcing a molecular clock. I then used a likelihood-ratio test (Felsenstein 1988, 1993) to compare the likelihood scores of the two trees; no statistical difference between the likelihood scores would indicate that enforcement of a molecular clock is compatible with the data.

RESULTS

The sequences of the eight *Agapornis* species and of *Loriculus galgulus* are deposited in GenBank under accession numbers AF001324 to AF001332. Of the 622 bases included in the cytochrome-*b* fragment that was sequenced, 169 positions (27%) were variable, and 96 (15%) were parsimony-informative. Of these variable sites, 123 (73%) were located at third-codon positions; 33 (19%) and 13 (8%) occurred at the first and second positions, respectively. Because there was no intraspecific sequence variation, I used only a single sample per species for tree building. Uncorrected sequence divergence between *Agapornis* species ranged from 0.5% (*nigrigenis-lilianae* and *personata-fischeri*) to 12.7% (*nigrigenis-cana*; see Table 3), and differences between *Agapornis* and *L. galgulus* ranged from 13.3% (*L. galgulus-A. pullaria*) to 15.3% (*L. galgulus-A. taranta*).

Pairwise numbers of transitions (Ti) and transversions (Tv) showed a bias in favor of transitional changes. When all codon positions were considered, Ti:Tv ratios within the *Loriculus/Agapornis* data set ranged from 6.00 (*A. personata-A. lilianae*) down to 1.14 (*L. galgulus-A. roseicollis*). If *Geopsittacus* was included in the comparisons, ratios were as low as 0.93 (*Geopsittacus-A. roseicollis*). This indicates that the deeper comparisons among these taxa will be affected by saturation due to multiple hits. Plots of transitions and transversions show that for this dataset, first position Ti and Tv increase linearly with increasing overall sequence divergence, without showing signs of saturation (Fig. 1A). However, at the third position, Ti changes reach saturation for comparisons of

TABLE 3. Pairwise distances between taxa over a 622-bp fragment of the cytochrome-*b* gene. Above the diagonal, uncorrected divergence; below the diagonal, maximum-likelihood distances calculated using the DNAML program in PHYLIP 3.5 (Felsenstein 1993).

	1	2	3	4	5	6	7	8	9	10
1 *L. galgulus*	—	0.1367	0.1334	0.1527	0.1383	0.1383	0.1447	0.1350	0.1399	0.1672
2 *A. personata*	0.1864	—	0.1061	0.1061	0.0096	0.0048	0.1579	0.0113	0.1206	0.1752
3 *A. pullaria*	0.1796	0.1313	—	0.0981	0.1109	0.1077	0.1061	0.1093	0.1141	0.1672
4 *A. taranta*	0.1946	0.1463	0.1070	—	0.1141	0.1061	0.1109	0.1125	0.1206	0.1704
5 *A. nigrigenis*	0.1917	0.0098	0.1366	0.1517	—	0.0113	0.0627	0.0048	0.1270	0.1801
6 *A. fischeri*	0.1880	0.0048	0.1329	0.1480	0.0115	—	0.0595	0.0129	0.1238	0.1768
7 *A. roseicollis*	0.1876	0.0606	0.1324	0.1475	0.0660	0.0623	—	0.0611	0.1238	0.1736
8 *A. lilianae*	0.1934	0.0115	0.1383	0.1534	0.0048	0.0131	0.0676	—	0.1254	0.1768
9 *A. cana*	0.1739	0.1475	0.1408	0.1558	0.1528	0.1491	0.1486	0.1545	—	0.1865
10 *Geopsittacus*	0.1995	0.2367	0.2297	0.2449	0.2420	0.2383	0.2379	0.2437	0.2242	—

taxa whose sequences differ by more than approximately 10% (Fig. 1B). Therefore, down-weighting third positions for parsimony analyses (as described in the Methods), or using a likelihood model that incorporates rate heterogeneity, should better reflect the phylogenetic information content of changes at the different codon positions. The disproportionately high number of changes occurring at the third position indicates that the region amplified and sequenced probably is in the cytochrome-*b* coding region (as intended) and is not a nuclear pseudogene (Arctander 1995). This is further supported by the fact that there were no insertions or deletions in the sequences relative to other known sequences (e.g. Leeton et al. 1994), and that when translated to an amino-acid sequence, no stop or nonsense codons were found.

An exhaustive parsimony search found four equally parsimonious trees, and a strict consensus of these was identical to the bootstrap tree shown in Figure 2A, except that *taranta* and *pullaria* formed a clade. Parsimony analysis of the weighted data produced a tree of similar topology but of improved resolution and better bootstrap support (Fig. 2B). Maximum-likelihood trees, reconstructed assuming either uniform or Gamma distributed rates, had the same topologies but differed in their likelihood scores (uniform rate: ln L = −2324.94; Gamma distributed rate: ln L = −2258.31). The trees differed only slightly from the parsimony tree in Figure 2A in their grouping of the white eye ring group: *personata* and *fischeri* were paired in the likelihood tree (66 and 54% reliability values for uniform and Gamma distributed rates, respectively) and placed as a sister group to the *nigrigenis-lilianae* clade found in the parsimony tree (98 and 85% reliability values). Support for the *cana-taranta* clade was better (87% reliability value) when uniform rates were assumed than when a Gamma distribution was used (50% reliability value), but support for the *roseicollis* and the white eye ring group nodes was 100% under both likelihood models.

In all of the tree searches that were done, the best trees grouped the white eye ring forms as a clade, with *roseicollis* at its base. Bootstrap and jackknife analyses, as well as quartet puzzling, showed that the white eye ring clade and the position of *roseicollis* are well supported using both parsimony and likelihood algorithms, with bootstrap/jackknife and reliability values of 99 to 100%. Also consistent was the close association between *nigrigenis* and *lilianae,* which was supported with bootstrap/jackknife and reliability values of at least 85%. All trees had *cana, taranta,* and *pullaria* at their bases but differed in their placements relative to each other.

Rooting trees with *Loriculus* and *Geopsittacus* produced nearly the same topology within *Agapornis.* Using equally weighted data, the use of *Loriculus* as an outgroup provided better resolution within the *Agapornis* clade, but the inclusion of *Geopsittacus* improved the resolution when weighted data were used in the analysis. This is not surprising, because sequence differences between *Geopsittacus* and the other species used in this analysis range from 16.7 to 18.7% (Table 3), and so third-codon position changes are likely to be affected by saturation (see Fig. 1).

The basal position of *cana,* which is endemic to Madagascar, suggests that it diverged from its congeners early in the history of the genus. A molecular "clock" calibration for cranes (Krajewski and King 1996), which is based on

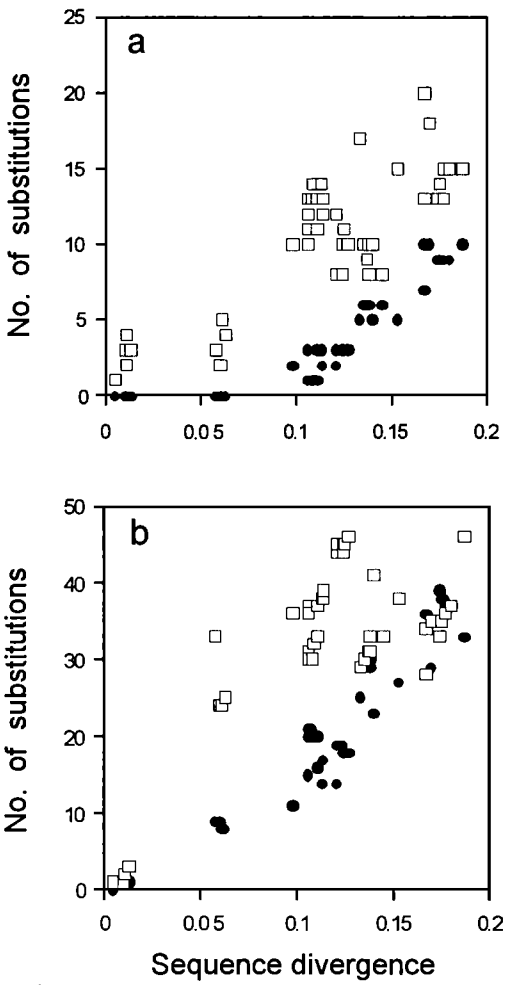

FIG. 1. Numbers of transition (Ti) and transversion (Tv) substitutions plotted against total sequence divergence for all species pairs included in Table 3. Shown are plots of Ti and Tv occurring at codon first positions (A) and third positions (B).

maximum-likelihood distances, was used to estimate the date of *cana*'s divergence from its closest relatives, *taranta* and *pullaria*. The comparison of likelihood scores for trees built with and without a molecular clock (ln L = −2328.65 and ln L = −2324.98, respectively) shows that they did not differ significantly (likelihood-ratio test, $0.5 < P < 0.9$), indicating that a molecular clock holds for these data. According to the crane calibration, the maximum rate of cytochrome-*b* sequence divergence (using maximum-likelihood distances; Table 3) is 0.7 to 1.7% per million years. The mean of the *cana-taranta* and *cana-pullaria* distances is 0.1483, so their distance from a common ancestor is estimated to be 0.0742. According to the crane calibration, this indicates that *cana* began diverging from its mainland relatives approximately 4.4 to 10.6 million years ago (MYA).

All of the trees generated from the sequence data are consistent with Dilger's (1960, 1961) grouping and with the characters used by Moreau (1948) to divide the genus into two groups. The phylogenies lend some support to Moreau's suggestion that *roseicollis* belongs to a lineage that is separate from the Group B species, but they also show that *roseicollis* is most closely related to the white eye ring forms, and that they all shared a relatively recent common ancestor. All trees place *roseicollis* and the white eye ring forms in a monophyletic clade, with *roseicollis* ancestral to the others. This segment of the tree also is the most robust, as indicated by the consistently high bootstrap values.

When nest types are mapped onto the tree, the construction of a cup nest appears on the tree with *roseicollis*, immediately preceding the origin of domed nest building (Fig. 2). Use of nest lining is ancestral in the group, and burrowing in arboreal termite nests is derived from nesting in a lined cavity. Alternative branching patterns, in which *roseicollis* was moved to different sections of a maximum-likelihood tree, were compared to the optimal tree using Kishino-Hasegawa tests. Forcing *roseicollis* to fall within the white eye ring clade resulted in a log-likelihood score that was significantly worse than that of the optimal tree (Kishino-Hasegawa test, $P = 0.0043$). Placing *roseicollis* in the *taranta/pullaria* clade also was a significantly unlikely arrangement given the data (Kishino-Hasegawa test, $P = 0.0001$).

DISCUSSION

The phylogenetic hypothesis presented here is consistent with the arrangement proposed by Dilger (1960). The white eye ring forms (*personata, fischeri, lilianae,* and *nigrigenis*) and *roseicollis* clearly compose a monophyletic clade. *Agapornis roseicollis* is at the base of the clade, and thus shared a common ancestor with the other four species. The percentage of sequence divergence between *personata, fischeri, lilianae,* and *nigrigenis* is much lower (range 0.5 to 1.1%) than the divergence between other species in

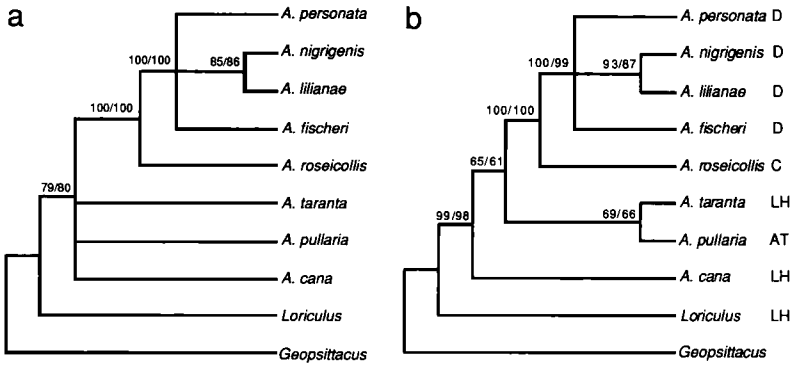

FIG. 2. Maximum-parsimony bootstrap trees found using PAUP* (test version 4.0.0d59, provided by D. L. Swofford). Numbers above branches are bootstrap values (2,000 replicates), followed by jackknife values (2,000 replicates). For each tree, the treelength (see text), consistency index (CI), and retention index (RI) are given. All tree statistics were calculated with uninformative characters excluded. (A) Maximum-parsimony tree found using equally weighted data. Treelength = 173, CI = 0.72, and RI = 0.75. (B) Maximum-parsimony tree found using weighted data (see text). Treelength = 328, CI = 0.67, and RI = 0.70. Letters next to species names indicate the type of nest used or built by that taxon: LH (lined hole), C (cup nest), D (domed nest), or AT (burrow in arboreal termitarium).

the genus (range 6.0 to 12.7%), indicating that these four species should be considered subspecies of *A. personata*. This supports the grouping advocated by Dilger (1960). Moreau (1948) also recognized the close relationship among the white eye ring forms, but he did not recommend combining them into one species.

The slightly closer relationship between *nigrigenis* and *lilianae* was first noted by Moreau (1948), and this relationship is supported by the mtDNA data presented here. As Moreau (1948) pointed out, the separation between the *nigrigenis/lilianae* and *personata/fischeri* clades probably was caused by mountain building at the head of Lake Nyasa. The northern end of the *personata/fischeri* division coincides with the location of the Rift Valley, which may be responsible for their separation; however, at the southern end of the division, no major geographic barriers separate the members within the two species pairs (Moreau 1948). Moreau (1948) suggested that the separation is maintained by regions of "miyombo" woodland, and, in the case of *nigrigenis* and *lilianae*, by a region of high elevation. The mtDNA phylogenies also show a close relationship between *taranta* and *pullaria*, which was noted by both Moreau (1948) and Dilger (1960).

The basal position of *cana* indicates that it probably became isolated on Madagascar relatively early in the course of diversification of the genus *Agapornis*. However, the amount of sequence divergence between *cana* and its closest mainland relatives (*taranta* and *pullaria*) suggests that colonization of Madagascar occurred after the island's separation from mainland Africa. According to a molecular clock calibration for cranes (Krajewski and King 1996), *cana* is estimated to have been diverging from other members of its genus for about 5 to 10 million years. Madagascar completed its separation from Africa some 121 MYA (Rabinowitz et al. 1983), long before *cana* began to diverge from other members of its genus. This indicates that this species did not diverge due to vicariance, but colonized an already insular Madagascar and thereafter was isolated from its congeners.

When the nest-type character is mapped onto the phylogeny (Fig. 2B), a reconstruction of the evolution of nest building in *Agapornis* supports the nest-lining hypothesis (see Introduction). In the genus, the habit of lining the nest cavity is ancestral to the use of nesting material for construction of a nest. Also, the four species that build domed nests are the most recently derived in the group and shared a common ancestor with *roseicollis*, which builds a simpler cup nest. This is consistent with the nest-lining hypothesis, which proposes that the construction of a domed nest evolved as the nest material initially used to line the nest cavity was used to construct progressively more complex nests. Another possibility that is

equally parsimonious given these data is that cup nests and domed nests evolved separately.

Alternative topologies that would lend less support to the nest-lining hypothesis are not likely given the sequence data. For example, placing *roseicollis* within the white eye ring group, which would not reflect the gradual elaboration of nest building predicted by the nest-lining hypothesis, is significantly unlikely. If this arrangement were correct, it would suggest that the cup nest built by *roseicollis* is derived from the domed nest. Putting *roseicollis* in the *cana/taranta/pullaria* clade, which would imply two clearly independent origins of nest building and again would fail to reflect the gradual elaboration of nest building, also is inconsistent with the sequence data.

It should be noted that within *Agapornis*, there is a correlation between nest adoption and nest construction: both *roseicollis* and *lilianae* frequently use the nests of colonial weaver birds (Forshaw 1989), and *fischeri* and *personata* appear to use other birds' nests (Moreau 1948). This is consistent with the nest-adoption hypothesis, but the data more strongly support the nest-lining hypothesis by indicating a progression from nest-lining behavior toward more elaborate building behavior. The observed correlation between nest construction and nest adoption in some *Agapornis* could occur if a bird that constructs a nest might be more likely to recognize and accept the nest of other species as a breeding site.

The construction of a nest within a cavity may be advantageous because it allows birds to modify cavities that might otherwise be unsuitable for breeding (Eberhard 1997). Observations of captive *A. personata* indicate that nest-construction behavior is flexible, and that a function of nest building is the modification of cavities (Vriends 1978). Alternatively, lovebirds would be expected to build a nest that was essentially the same, regardless of the size or shape of the cavity. Vriends (1978) found that if the nest-box opening is too large, birds sometimes pile nesting material up against it to make the entrance smaller. He also noted that the extent of nest construction depends on the size of the cavity—if space is limited, the dome may be omitted. Vriends also observed that females will add extra material to their nests if light leaks into the nest chamber, or if there are any sharp projections in the nest interior.

An important difference between the *Agapornis* species that construct nests and those that do not is breeding density. Those that build nests (i.e. the white eye ring forms and *roseicollis*) are colonial, whereas the others are solitary breeders. In captivity, breeding pairs of *cana* and *taranta* are extremely territorial and must be kept in separate cages (Vriends 1978, Erhart 1983). Gregarious breeding may have been facilitated by the evolution of nest building, because the ability to construct a nest could give breeding pairs flexibility in their choice of nesting sites (Eberhard 1997). An association between increasingly complex nest construction and gregarious nesting also has been shown in a phylogenetic analysis of the evolution of nest construction in swallows (Winkler and Sheldon 1993). In the case of swallows, Winkler and Sheldon suggest that the transition from cup nests to domed nests permitted higher breeding densities by reducing forced extrapair copulation attempts. In *Agapornis*, the shift from sexual dimorphism (in the "primitive" species) to sexual monomorphism (in *roseicollis* and the white eye ring forms) may be a result of the shift to colonial breeding (Dilger 1960), perhaps because of nonsexual social selection on plumage involved in signaling (which is likely to occur more frequently in a colony) by both sexes (West-Eberhard 1983).

A complete assessment of the adaptive value of nest building and colonial breeding clearly is an ecological question that involves knowledge about the habitat in which these species live (and have evolved), the availability of nest cavities, and quantification of the costs and benefits associated with gregarious breeding. Field studies of *Agapornis* are lacking and are necessary to evaluate the hypotheses proposed here regarding the adaptive value and evolution of nest-building behavior in the genus. In particular, data on the availability and distribution of nest cavities, and the lovebirds' pattern of occupation of available cavities, could be used to test the hypothesis that the construction of nests within cavities facilitates gregarious breeding.

The phylogenetic data presented here support a historical reconstruction of the evolution of nest-building behavior in which the construction of a nest within a cavity is derived from the habit of lining the nest. Several of the

differences traditionally used to distinguish the nest-building species from the "primitive" species probably are linked with the change in nesting behavior. One of these is the method of carrying nesting material—the change from carrying material in the feathers to carrying it in the beak permitted transport of larger and/or heavier items. Plumage changes may have followed changes in nest-building behavior, because the switch from dimorphism to monomorphism probably is linked to the shift from solitary to gregarious breeding. It is likely that gregarious breeding itself was facilitated by the ability to construct nest structures within cavities, because the ability to modify unsuitable cavities would give breeding pairs increased flexibility in nest placement.

ACKNOWLEDGMENTS

I thank H. Hollocher for providing me with a lab in which to work; she and D. Kutzler patiently taught me PCR and sequencing techniques. P. Wade provided an easy and reliable feather-extraction protocol; J. Rowden and K. Petren kindly gave me the primers that were used for PCR and sequencing. Feather samples were provided by a number of people: C. Falzone (Brookfield Zoo), M. Mitchell (Dickerson Park Zoo), J. Landvater and K. Raby (San Francisco Zoo), D. Rimlinger (San Diego Zoo), C. Siegel (Dallas Zoo), M. Weldon (Fort Wayne Zoo), and D. Emlen and K. Bright; A. Lyles helped me to locate zoos with *Agapornis* and *Loriculus* in their collections. While working on this project, I was supported by Princeton University; funding for the laboratory work was provided by the Association for Women in Science Educational Foundation and Princeton University. The manuscript benefitted from the comments of P. Grant, H. Hollocher, A. Dobson, H. Horn, M. Bjvrklund, and R. Gray.

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Associate Editor: A. J. Baker