



A molecular phylogeny of the peacock-pheasants (Galliformes: *Polyplectron* spp.) indicates loss and reduction of ornamental traits and display behaviours

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The South-east Asian pheasant genus *Polyplectron* is comprised of six or seven species which are characterized by ocelli (ornamental eye-spots) in all but one species, though the sizes and distribution of ocelli vary among species. All *Polyplectron* species have lateral displays, but species with ocelli also display frontally to females, with feathers held erect and spread to clearly display the ocelli. The two least ornamented *Polyplectron* species, one of which completely lacks ocelli, have been considered the primitive members of the genus, implying that ocelli are derived. We examined this hypothesis phylogenetically using complete mitochondrial cytochrome *b* and control region sequences, as well as sequences from intron G in the nuclear ovomucoid gene, and found that the two least ornamented species are in fact the most recently evolved. Thus, the absence and reduction of ocelli and other ornamental traits in *Polyplectron* are recent losses. The only variable that may correlate with the reduction in ornamentation is habitat, as the two less-ornamented species inhabit montane regions, while the ornamented species inhabit lowland regions. The implications of these findings are discussed in light of models of sexual selection. The phylogeny is not congruent with current geographical distributions, and there is little evidence that Pleistocene sea level changes promoted speciation in this genus. Maximum likelihood and maximum parsimony analyses of cytochrome *b* sequences suggest that the closest relatives of *Polyplectron* are probably the peafowl and the argus pheasants.

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INTRODUCTION

The peacock-pheasants, *Polyplectron* spp., are comprised of six or seven species of small pheasants distributed in South-east Asia (Fig. 1). Similar to the true peafowl (*Pavo* spp.), males of all but one species of *Polyplectron* have ocelli (eye spots) on feathers which are displayed frontally to females during courtship (Table 1). Unlike the peafowl, which have ocelli only on highly specialized tail coverts, the ocelli of *Polyplectron*

occur on many different feather types including tail, tail coverts, flight feathers, wing coverts and the mantle. The size, colour and distribution of ocelli on different feathers vary among species. Other ornamental traits include crests and bare patches of facial skin which are present in some species. Sexual dimorphism is moderate, with females having smaller and less iridescent ocelli than those of males.

Phylogenetic relationships among *Polyplectron* have not been well examined. Earlier authors (Beebe, 1914; Verheyen, 1956) separated *Polyplectron* into two genera: *Chalcurus* contained the two least-ornamented species, *chalcurum* (ocelli absent), and *inopinatum*

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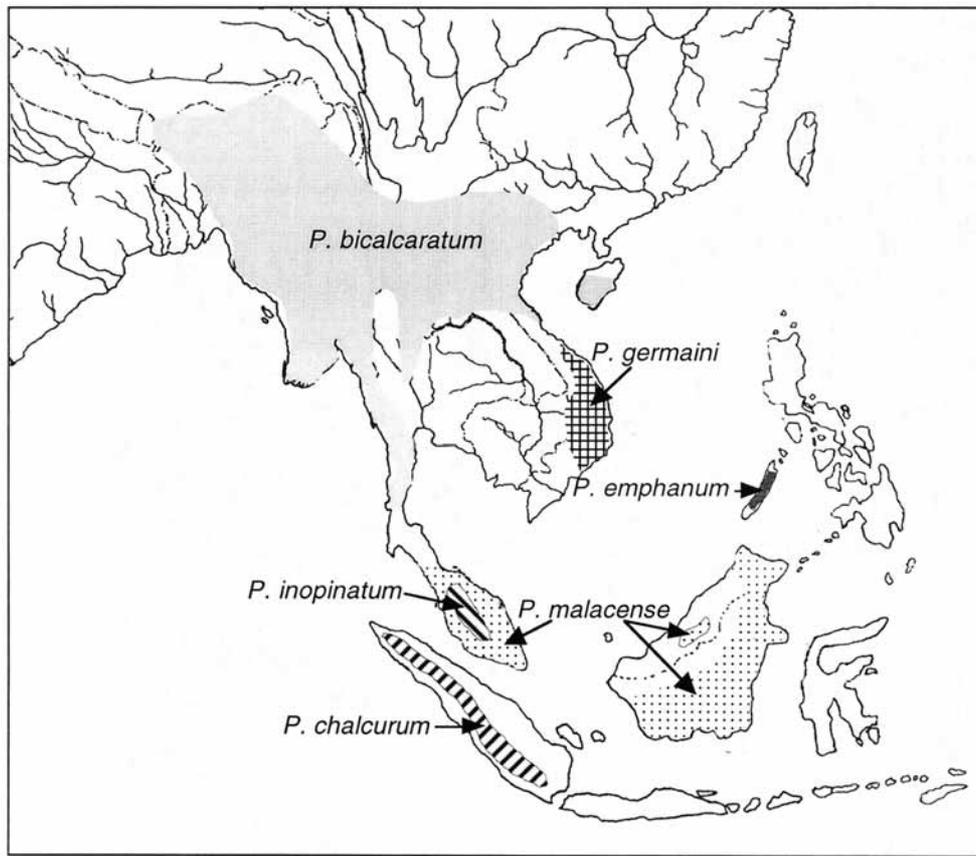


Figure 1. Known distribution of *Polyplectron* species (from Johnsgard, 1999).

(ocelli small and simple), while *Polyplectron* contained the more ornamented species. Beebe (1918–1922) listed eight traits which separated *Chalcurus* and *Polyplectron* (e.g. Fig. 2A). Four of these are ornamental traits, three refer to coloration of non-ornamental plumage, and the final trait is the degree of sexual dimorphism. Later authors (e.g. Delacour, 1977; Johnsgard, 1999) combined the two genera. Johnsgard (1999: 310) referred to *P. chalcurum* and *P. inopinatum* as 'generalized', and considered *P. inopinatum* as intermediate between *P. chalcurum* and the other *Polyplectron* species. The evolutionary pathway proposed by Johnsgard (1999) assumes that ocelli evolved after *P. chalcurum* separated from the other *Polyplectron* species and then underwent further elaboration in the more recently evolved taxa. This pattern is consistent with sexual selection theories which tend to emphasize the evolution and continued elaboration of ornamental traits (e.g. Ryan & Keddy-Hector, 1992; Andersson, 1994). However, recent phylogenetic studies have demonstrated that loss of dichromatism or specific male ornamental traits may be common (Peterson, 1996; Price & Birch, 1996; Omland, 1997; Wiens, 1999), indicating that it may be

erroneous to consider more elaborate ornamentation as the derived condition without a well-supported phylogeny.

A phylogeny of *Polyplectron* could also assist in understanding the biogeography of the Sunda region, particularly to determine whether Pleistocene sea level changes have increased isolation of populations (leading to speciation) or affected the distribution of species in this region. Several species are distributed primarily in Indochina (*P. bicalcaratum* and *P. germaini*), while others are island endemics (*P. chalcurum* and *P. emphanum*). *P. malacense* occurs on Borneo (*P. m. schleiermacheri*; sometimes classified as *P. schleiermacheri* Brüggemann, e.g. Johnsgard, 1999) and the lowlands of the Malay Peninsula (*P. m. malacense*) where it is ecologically separated from *P. inopinatum* which inhabits high montane regions of the Malay Peninsula (Fig. 1).

The relationship of *Polyplectron* to other phasianids is also poorly understood. *Polyplectron* has usually been allied with *Argusianus argus*, which has ocelli, and *Rheinartia ocellata*, which lacks ocelli (Beebe, 1914; Verheyen, 1956; Delacour, 1977; Johnsgard, 1999). The true peafowl, *Pavo* spp., which have ocelli,

Table 1. Pavonine species (common names in parentheses), presence or absence of facial ornaments, ocelli and frontal display behaviour. Data are from Johnsgard (1999) and references therein

Species	Crest ¹	Facial skin ²	Ocelli ¹	Comments	Display ³
<i>Polyplectron</i> , peacock-pheasants					
<i>P. emphanum</i> Temminck (Palawan)	+	Red	+	Restricted to tail	L, F ⁴
<i>P. malacense</i> Scopoli (Malay)	+	Orange	+	Ocelli widespread	L, F
<i>P. germaini</i> Elliot (Germain's)	+	Red	+	Ocelli widespread	L, F
<i>P. bicalcaratum</i> Linnaeus (grey)	+	Yellow or Red	+	Ocelli widespread	L, F
<i>P. inopinatum</i> Rothschild (Rothschild's)	+	–	+	Ocelli small, simple	L, F
<i>P. chalcureum</i> Lesson (bronze-tailed)	–	–	–	Iridescence on tail	L
Other pavonine species					
<i>Pavo cristatus</i> Linnaeus (Indian peafowl)	+	White	+	On specialized tail coverts	F
<i>P. muticus</i> Linné (green peafowl)	+	Blue, Yellow	+	On specialized tail coverts	F
<i>Afropavo congensis</i> Chapin (Congo peafowl)	+	Blue-grey	–	Possible vestigial ocelli	L, F
<i>Argusianus argus</i> Linnaeus (great argus)	+	Blue	+	On specialized wing feathers	L, F
<i>Rheinartia ocellata</i> Elliot (crested argus)	+	–	–	Simple spots on body	L

¹ +, present; –, absent.

² Colour of facial skin, if present.

³ L, lateral display; F, frontally oriented display.

⁴ In *P. emphanum*, the body is lateral to the female during the frontally oriented display, though the ocelli are displayed in a manner similar to a true frontal display.

and the Congo peafowl, *Afropavo congensis*, which lacks ocelli, have also been suggested as relatives of *Polyplectron* (Johnsgard, 1999), though not by all authors (Beebe, 1914; Verheyen, 1956). Recent analyses, using molecular sequence data, did not clearly resolve the relationships between *Polyplectron* and their hypothesized relatives (Akishinonomiya *et al.*, 1995; Kimball, Braun & Ligon, 1997; Kimball *et al.*, 1999), though these studies provided some evidence supporting the placement of *Polyplectron* in a larger clade with *Argusianus*, *Pavo* and *Afropavo*.

We used complete mitochondrial cytochrome *b* (CYB) and control region (CR) sequences, as well as sequences from intron G of the nuclear ovomucoid gene (OVOG) to examine four main questions: (1) What are the evolutionary relationships within *Polyplectron*? (2) Did Pleistocene sea level changes affect speciation and species distributions in *Polyplectron*? (3) What is the evolutionary pathway of the display behaviours and ocelli in *Polyplectron*? and (4) Which are the closest relatives of *Polyplectron*?

METHODS

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

We extracted DNA from blood or feathers, PCR amplified complete CYB, CR and OVOG fragments, and used automated sequencing to obtain double-stranded sequences as described elsewhere (Randi & Lucchini, 1998; Kimball *et al.*, 1999; Armstrong, Braun & Kimball, in press).

SEQUENCE ALIGNMENT AND TAXON SELECTION

CYB and CR sequences were obtained from six species of *Polyplectron* and the putative relatives (*Pavo cristatus*, *P. muticus*, *Afropavo congensis*, *Argusianus argus* and *Rheinartia ocellata*; see Johnsgard, 1999 for justification); OVOG sequences were obtained from all but *Rheinartia*. To determine the relationship of *Polyplectron* to other galliforms, and whether the closest relatives of *Polyplectron* are *Pavo*, *Afropavo*, *Argusianus* and *Rheinartia*, the CYB sequences of *Polyplectron* were compared with a large data set (46 taxa) of available galliform

CYB sequences (Kimball *et al.*, 1999; Shen, Tsudzuki & Nakamura, 1999; Randi *et al.*, 2000).

Sequences were aligned as described previously (Randi & Lucchini, 1998; Kimball *et al.*, 1999; Armstrong *et al.*, in press). For the CR, one region was difficult to align with the outgroups and was removed from analyses. When compared with the control region from the complete mitochondrion of *Gallus gallus*, these sites correspond to nucleotides 213 to 247 in the hypervariable part of domain I. Sequences new to this study have been deposited in GenBank (Accessions AF330060–AF330065, AF331954–AF331959, AJ295255–AJ295260, AJ309513–AJ309517).

PHYLOGENETIC ANALYSES

Phylogenetic analyses were performed using PAUP* 4.0b2 (Swofford, 1999). To compare rates of divergence among the different molecular partitions, *p*-distances were estimated separately for CYB, CR and OVOG. CR and OVOG *p*-distances were divided by CYB *p*-distances, and these ratios were averaged across all comparisons.

To examine whether the CYB data was clock-like we used the likelihood ratio test for a molecular clock (Huelsenbeck & Crandall, 1997). To estimate divergence times, branch lengths from this CYB maximum likelihood (ML) analyses were used, and a sequence divergence rate of 2% per Myr was assumed (Klicka & Zink, 1997; Avise & Walker, 1998). Confidence intervals presented with molecular clock estimates were calculated assuming a Poisson process of substitution. Since the variance of a Poisson process is equal to the mean number of events (substitutions), 95% confidence intervals are twice the square-root of the expected number of substitutions along the relevant branch.

The most parsimonious (MP) trees were identified using equally weighted parsimony with branch-and-bound searches for the small CYB, CR and OVOG alignments. The reliability of specific groupings in parsimony analyses was assessed using 1000 bootstrap replicates and branch-and-bound searches. For CR and OVOG, gaps were both treated as missing data as well as coded using the simple indel coding method of Simmons & Ochoterena (2000).

To examine relationships between *Polyplectron* and other galliforms, we analysed the large (46 taxa) CYB data set. As homoplasy was possible among the more divergent taxa in this data set, we examined several types of weighting for parsimony analyses involving the large data set, in addition to equally weighted parsimony. These included (1) successive reweighting using the maximum values of both the rescaled consistency index and the consistency index (e.g. Farris, 1969; Carpenter, 1988); (2) removal of all third positions (e.g. Kornegay *et al.*, 1993); (3) exclusion of transitions at third positions (e.g. Milinkovitch *et al.*,

1996); and (4) transversions only (e.g. Mindell & Thacker, 1996). For equally weighted parsimony, and each of the different weighting schemes, we found the MP trees using heuristic searches with 100 random addition sequence replicates. To assess reliability at specific nodes, we performed 500 bootstrap replicates using heuristic searches with 10 random sequence addition replicates per bootstrap replicate.

To test for congruence among the CYB, CR and OVOG partitions, we used the partition homogeneity test (incongruence length difference test; Farris *et al.*, 1995) in PAUP*, with 1000 replicates and branch-and-bound searches using both all sites and informative sites only.

Models of DNA substitution and parameter estimates for ML analyses were determined using the hierarchical likelihood ratio test (Huelsenbeck & Crandall, 1997). Among-site rate heterogeneity was accommodated using a four-category discrete approximation to a Γ -distribution (Yang, 1994).

To test specific hypotheses regarding evolution within *Polyplectron*, the difference in likelihood of the ML topology and several alternative topologies (used as the null hypotheses) were examined using the SOWH test (Swofford *et al.*, 1996; Goldman, Anderson & Rodrigo, 2000). This test compares a test statistic (2δ , twice the difference in likelihood of the ML tree and a distinct topology) to a null distribution generated by simulation. For each test, 100 simulated data sets based on the 11-taxon CYB+CR combined data were generated using Seq-Gen 1.1 (Rambaut & Grassly, 1997) and the ML tree from each simulated data set was identified. The 2δ test statistic was generated from each simulated data set, establishing the null distribution for this statistic. Parameter estimates for each simulated data set were initially obtained from a neighbour joining tree of HKY85 (Hasegawa, Kishino & Yano, 1985) distances, followed by one successive iteration using ML as described above.

MORPHOLOGICAL TRAITS

We used data available in Delacour (1977) to examine changes in body size and the degree of sexual size dimorphism. Delacour provided a size range of tarsus, wing and tail length for each species and sex, and we used the midpoint of this range. Use of either the largest or smallest value led to similar conclusions, and our results were very similar to those of Davison (1985a) for similar analyses (see below).

RESULTS AND DISCUSSION

MOLECULAR EVOLUTION OF CYB, CR AND OVOG

All CYB sequences contained an open reading frame that could encode a protein with significant identity

to other CYB proteins. The haem-ligating histidines and other conserved residues (Howell, 1989) could be identified, suggesting the sequences were functional CYB genes rather than numts (nuclear pseudogenes). The mitochondrial origin of the CYB sequence of *P. bicalcaratum* has been confirmed both by sequencing PCR products amplified from muscle tissue, which is rich in mitochondria, and through Southern blot analysis (see Kimball *et al.*, 1999). When nuclear copies are present, the nuclear and mitochondrial products are often co-amplified by PCR, and sequences of these products will exhibit ambiguities at some positions (Sorenson & Fleischer, 1996; Sorenson & Quinn, 1998). All electropherograms appeared to represent only a single amplified product. This, combined with the congruence obtained between the data sets (see below) suggested that these sequences were unlikely to represent nuclear pseudogenes.

The CYB alignment contained 1143 sites with no indels. The small CYB alignment (11 taxa) had 308 variable and 203 parsimony informative sites. The best model was HKY85 with a Γ -distribution to accommodate site-to-site rate heterogeneity. The transition/transversion ratio = 7.70, and the estimated shape parameter (α) for a Γ -distribution was 0.16, indicating high among-site rate heterogeneity.

The CR alignment (11 taxa) contained 1152 sites, with 227 variable and 179 parsimony informative sites. With the gap matrix there were 1205 sites, of which 280 were variable and 218 were parsimony informative. The best model was HKY85 with a Γ -distribution. The transition/transversion ratio = 3.15, and the estimated shape parameter = 0.09.

The OVOG data (10 taxa) had 471 sites, with 56 variable and 21 parsimony informative sites. With the gap matrix, there were 477 sites, of which 62 were variable and 26 parsimony informative. The best model for OVOG was HKY85 and the estimated transition/transversion ratio = 3.21.

The large CYB alignment (46 taxa) had 541 variable and 457 parsimony informative sites. The best model was GTR with both invariant sites and a Γ -distribution. The estimated proportion of invariant sites = 0.48 and the estimated shape parameter = 1.04. The rate parameter of the most slowly accumulating transitions (A–G) was about six times that of the fastest class of transversions (A–C).

The three molecular partitions (CYB, CR and OVOG), using all taxa except *Rheinartia*, represented congruent data partitions (all sites: $P=0.63$; informative sites only: $P=0.61$). Including *Rheinartia*, CYB and CR were also congruent (all sites: $P=0.33$; informative sites only: $P=0.33$). Thus, we analysed both combined and individual data sets.

The rate of CYB evolution was compared with that

of CR and OVOG, revealing that the CR evolved approximately 82% as rapidly as CYB while OVOG evolved approximately 35% as rapidly as CYB. The faster evolving domains I and III of the CR and the more slowly evolving domain II (Randi & Lucchini, 1998) were compared separately to CYB. The evolutionary rates of domains I and III were 94% and 124% of CYB, respectively, while domain II evolved about 41% as fast as CYB. These data support several recent studies (e.g. Randi & Lucchini, 1998) which demonstrate that the degree of sequence divergence in CR is not very different from that of CYB. Even the hypervariable domains I and III were less divergent or only slightly faster than CYB, and both were much lower than the ten-fold difference in evolutionary rates that has been suggested for the hypervariable domains of the CR in birds (e.g. Avise & Walker, 1998). The lower degree of sequence divergence in the CR is also reflected in the phylogenetic resolution among the more recently evolved species in *Polyplectron*, where branch lengths were short (data not shown) and bootstrap support for the CR was lower than for CYB (Fig. 2B), particularly when gaps were treated as missing data (data not shown).

RELATIONSHIPS WITHIN *POLYPLECTRON*

Polyplectron is a monophyletic genus with high levels of bootstrap support (Figs 2B, 3). Using the combined data (either including or excluding OVOG), the ML and single MP tree were identical topologically (Fig. 2B). MP analyses of the CR and ML analyses of CYB produced a topology identical to Figure 2B, though slight differences were observed in other analyses (e.g. see asterisks in Fig. 2B).

OVOG was congruent with the other partitions, but could not resolve relationships among the more recently evolved species. However, *P. bicalcaratum* and *P. chalcurum* had identical OVOG sequences while *P. inopinatum* and *P. germaini* differed from these two taxa by one and two bases, respectively, consistent with the topology in Figure 2B. In addition, the OVOG data set contained two indels that supported the topology in Figure 2B. A 6 bp deletion united *P. germaini*, *P. inopinatum*, *P. bicalcaratum* and *P. chalcurum* to the exclusion of the remaining *Polyplectron* and outgroup species; a second deletion of 7 bp united the four taxa above with *P. malacense*, to the exclusion of *P. emphanum* and the outgroup taxa. Taken as a whole, these results supported the hypothesis that *P. chalcurum* (in a clade with *P. bicalcaratum*; Fig. 2B) and *P. inopinatum* were recently evolved, though these two species have traditionally been considered the basal members of *Polyplectron* (e.g. Fig. 2A).

On the basis of the SOWH test, the ML tree (Fig. 2B) was significantly more likely ($2\delta=54.75$; 2δ -critical =

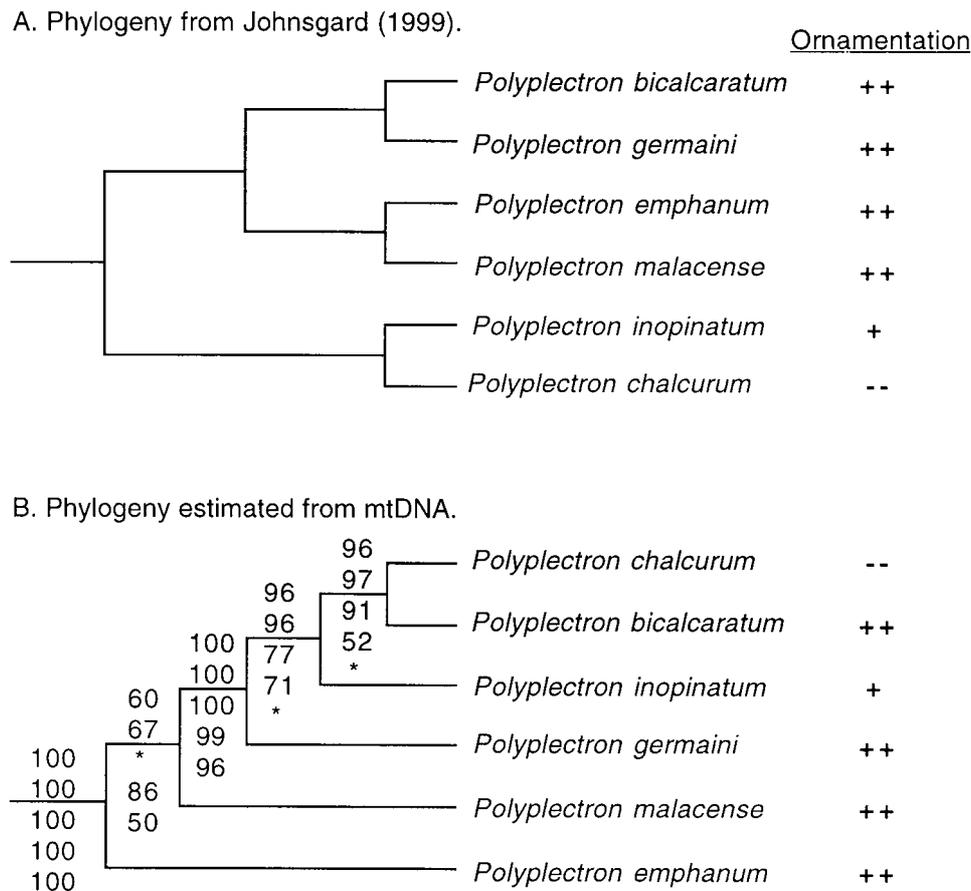


Figure 2. Phylogenetic relationships among *Polyplectron*. A, phylogenetic hypothesis presented as figure 1 in Johnsgard (1999). B, phylogeny estimated from ML and MP analyses of the combined data; rooted to outgroup taxa. Analyses using OVOG had one fewer outgroup taxa than CYB and CR analyses. Numbers at nodes indicate bootstrap support from equally weighted parsimony analysis (including gap matrices) for the CYB+CR+OVOG data (top), CYB+CR, CYB (middle), CR and OVOG (bottom). Asterisks indicate node not present in that analysis.

9.72; $P < 0.01$), than any tree in which the least ornamented taxa (*P. chalcurum* and *P. inopinatum*) were placed in a grade basal to the other *Polyplectron* species, a phylogeny suggested by Johnsgard's (1999: 310) description of *P. chalcurum* and *P. inopinatum* as 'generalized' with *P. inopinatum* intermediate between *P. chalcurum* and the other *Polyplectron* species. The SOWH test also indicated that the ML tree (Fig. 2B) was also significantly more likely ($2\delta = 56.77$; 2δ -critical = 9.56; $P < 0.01$) than any tree in which *P. chalcurum* and *P. inopinatum* were placed in a clade basal to other *Polyplectron* species (e.g. Fig. 2A), providing strong evidence against the former classification of these two species in a separate genus.

BIOGEOGRAPHY OF *POLYPLECTRON*

The phylogeny of *Polyplectron* showed little concordance with current geographical distributions (Figs

1, 2B). For example, the two species on the Malay peninsula, *P. malacense* and *P. inopinatum*, are not closely related to each other, which suggests that two separate colonizations of the Malay Peninsula occurred. To examine this hypothesis more rigorously, we used the SOWH test to determine whether our most likely tree (Fig. 2B), which separated the taxa, was significantly better than the best tree which assumed that *P. malacense* and *P. inopinatum* formed a clade. The results indicate that two colonizations of the Malay Peninsula were significantly more likely than one ($2\delta = 96.51$; 2δ -critical = 6.19; $P < 0.01$).

Since a molecular clock could not be rejected using the CYB data, we examined whether divergence times in *Polyplectron* were consistent with speciation events driven by Pleistocene sea level changes (e.g. Heaney, 1986). Our molecular clock suggests that *P. chalcurum* diverged from *P. bicalcaratum* approximately 0.7 (0.2–1.2) Mya, while *P. inopinatum* diverged from *P. bi-*

calcaratum approximately 1.5 (0.8–2.2) Mya. These dates suggest that, at most, only two speciation events in this genus occurred in the Pleistocene, with other speciation events occurring in the Pliocene. Of the two island endemics, only *P. chalcurum* (on Sumatra) may have arisen due to Pleistocene vicariant events. The absence of a strong influence of Pleistocene changes on speciation in this genus is consistent with several studies which suggest that sea level changes and other Pleistocene affects have had less effect on speciation of South-east Asian fauna than has previously been assumed (e.g. Hewitt, 2000).

EVOLUTION OF ORNAMENTS AND DISPLAYS

The most striking morphological feature of most *Polyplectron* species are the ocelli. In males, these complex structures contain a central iridescent region of one or more colours, outlined by a ring of dark and then light coloration, which makes the ocelli very prominent. In most species, ocelli are widely distributed on the body (Table 1). However, there are some exceptions. *P. emphanum* has ocelli on the tail and tail coverts, while the mantle and wing coverts lack ocelli and are instead an iridescent metallic blue colour. Although *P. inopinatum* has ocelli, they are fewer in number, smaller and less iridescent than in other ocellated species. Furthermore, the ocelli on the tail and tail coverts of *P. inopinatum* lack the lightly coloured ring, making them less prominent. Thus, *P. inopinatum* appears less ornamented than the other species, except for *P. chalcurum*, which lacks ocelli and has instead only small patches of iridescence on the tail.

Interestingly, the species of *Polyplectron* with the best developed ocelli also have other ornamental traits, such as crests and bare facial skin surrounding the eye (Table 1). The facial skin is brightly coloured in most species, and provides a contrast which emphasizes the eye. Similar to the pattern with ocelli, both *P. inopinatum* and *P. chalcurum* lack a crest and bare facial skin. Thus, compared with the other *Polyplectron* species, there appears to be an overall reduction in ornamentation of males in both of these recently evolved species.

Our results suggest that the presence of ocelli and other ornamental traits are primitive in *Polyplectron*, having been reduced and lost relatively recently (see molecular clock estimates above) in *P. inopinatum* and *P. chalcurum*. Our phylogeny (Fig. 2B) suggests two possible scenarios: either there has been independent selection for reduction of ornamentation in *P. chalcurum* and *P. inopinatum*, or there has been selection for ornament reduction in the ancestor of *P. chalcurum* and *P. inopinatum*, followed by a gain of ornamentation in *P. bicalcaratum*. If losses and gains are considered equally likely, it is not possible to distinguish which

scenario is more likely. However, loss of complex traits, such as dichromatism, has been suggested to be more likely than gains (Omland, 1997). Making the assumption that losses are more likely than gains, it is probable that there were two independent losses rather than a loss followed by a gain. We used the SOWH test to examine whether *P. chalcurum* and *P. inopinatum* might form a clade (to the exclusion of *P. bicalcaratum*), as would arise had there been a single selective event for a reduction in ornamentation (though the reduction is greater in *P. chalcurum*). The results suggest that the topology in Figure 2B is significantly more likely than any topology in which *P. chalcurum* and *P. inopinatum* form a clade ($2\delta = 33.32$; 2δ -critical = 6.61; $P < 0.01$), and suggest that two independent losses (or possibly one loss and one gain) are more likely than a single loss.

The display behaviours of most *Polyplectron* prominently exhibit the ocelli. Lateral displays, which are thought to be primitive among pheasants (Davison, 1980), are present in all *Polyplectron* species. In these displays, the near wing is lowered and spread, the far wing is raised, and the tail is spread and angled toward the female, displaying some ocelli (if present) to females (Johnsgard, 1999). Those species with ocelli also have a frontally oriented display (Table 1), though the frontal display of *P. inopinatum* has been seen only rarely (Johnsgard, 1999). In frontally oriented displays, the feathers with ocelli are spread, held erect and displayed forward and the female is presented a large surface area in which the maximum number of ocelli are shown. During these frontal displays, the head is held sideways providing the female with a view of one eye surrounded by ocelli. *P. chalcurum*, which lacks ocelli, also lacks a frontal display (Davison, 1985a), suggesting that displays and ocelli may be linked in this group. A coupling between the gain of displays and morphological traits, but a decoupling in their loss, has been suggested by some studies (Prum, 1990; Wiens, 2000). In contrast to these studies, our results suggest that loss of both display and morphological traits are linked in *Polyplectron*.

SEXUAL SELECTION AND THE LOSS AND REDUCTION OF ORNAMENTAL TRAITS

Information on sexual selection and mating systems are poorly known for *Polyplectron* (see Johnsgard, 1999 for a discussion). *P. malacense* has a polygynous or promiscuous mating system in which males maintain display sites in forest clearings (e.g. Davison, 1983a, b; McGowan, 1994), suggesting strong sexual selection in this species. Unfortunately, whether ocelli are important in male mating success is not known. Although not all avian ornamental traits are involved in sexual selection (e.g. Ligon, Kimball & Merola-Zwartjes,

1998), the display of the more ornamented *Polyplectron* species strongly emphasize the ocelli, making it likely that the ocelli, at least, are important in sexual selection in those *Polyplectron* species. In addition, the ocellated train of *Pavo*, which is also displayed prominently to females, is important in female choice (Petrie, Halliday & Sanders, 1991; Petrie & Halliday, 1994). At this time, there is no evidence that ornamentation in *Polyplectron* or any of its putative relatives is involved in male–male competition, in addition to or instead of female mate choice.

Although sexual selection is generally thought to select for larger or more elaborate ornaments and displays (e.g. Ryan & Keddy-Hector, 1992; Andersson, 1994), it is important to examine factors which may explain the loss and reduction of sexually selected traits. Several models have been proposed to explain changes in the targets of sexual selection (Gilliard, 1956; Møller & Pomiankowski, 1993; Schluter & Price, 1993; Hill, 1994b; Holland & Rice, 1998). Females may focus on new male traits for several reasons, such as when current male traits no longer convey non-cheatable information about male quality (e.g. Møller & Pomiankowski, 1993; Hill, 1994b; Ligon *et al.*, 1998); or if females cease to respond to existing male ornamentation (Holland & Rice, 1998). Female preferences may be lost, if, for example, choice of mate has no effect on female fitness.

There may also be selection on males to shift the target of female choice if it is possible to change to a less costly ornament or display (Gilliard, 1956; Kusmiński *et al.*, 1993; Johnson, 1999). Situations which may select for such a change include habitats which inhibit transmission of an existing signal (e.g. Schluter & Price, 1993; Endler & Basolo, 1998), limited or unavailable resources in the current habitat which are necessary for signal production (e.g. Hill, 1994a), or strong natural selection against the ornament (e.g. Endler, 1980). In addition, in some situations, genetic drift may also be important in the loss of either male traits or female preferences (e.g. Peterson, 1996).

We examined whether we could detect a shift in the targets of sexual selection in the two *Polyplectron* species which appeared to have reduced ornamentation. Such a change could have been from ocelli, to song, display behaviours, body size or an alternative ornamental trait. Descriptions of the song in *Polyplectron* do not suggest an increase in song complexity in *P. chalcurum* or *P. inopinatum* (Johnsgard, 1999). The loss of the frontal display in *P. chalcurum*, and the reduced emphasis on frontal displays in *P. inopinatum*, combined with the absence of novel or elaborated displays in either of these species, suggests that neither song nor display are likely to have become targets of female choice.

To determine whether there has been a shift towards

Table 2. Degree of size dimorphism (male/female) in *Polyplectron*. Taxa are organized phylogenetically, with the most recently evolved species at the top

Species	Tarsus	Wing	Tail
<i>P. chalcurum</i>	1.18	1.13	1.60
<i>P. bicalcaratum</i>	1.15	1.15	1.55
<i>P. inopinatum</i>	1.18	1.33	1.45
<i>P. germaini</i>	1.18	1.10	1.21
<i>P. malacense</i>	1.08	1.14	1.32
<i>P. emphanum</i>	1.20	1.12	1.46

Table 3. Ratios of male traits in *Polyplectron*. Taxa are organized phylogenetically, with the most recently evolved species at the top

Species	Tail/wing	Tail/tarsus	Wing/tarsus
<i>P. chalcurum</i>	1.82	4.92	2.71
<i>P. bicalcaratum</i>	1.67	4.84	2.90
<i>P. inopinatum</i>	1.48	5.54	3.73
<i>P. germaini</i>	1.50	4.38	2.92
<i>P. malacense</i>	1.18	3.77	3.19
<i>P. emphanum</i>	1.27	3.71	2.92

selection for proportionately larger body size in males, we examined the ratio of male to female tarsus and wing length (Table 2). It does not appear that there has been an increase in male body size, relative to females, in either *P. chalcurum* or *P. inopinatum* (Table 2), suggesting that body size dimorphism, as measured by tarsus and wing length, has not increased in either of these species.

Davison (1985a) suggests that, in *P. chalcurum*, tail length may be the target of sexual selection. He collected body measurements from all of the *Polyplectron* species and made two observations. First, the range in tail lengths was greatest in *P. chalcurum*. Second, he divided maximum tail length by wing length (to correct for body size) and observed that *P. chalcurum* had a longer tail than the other *Polyplectron* species (Davison, 1985a). Using the values in Delacour (1977), we obtain very similar values to that of Davison (1985a; Table 3). When considered in a phylogenetic context (Fig. 2B), the ratio of tail to wing length appears to be increasing in the more recently evolved species, such that both *P. chalcurum* and *P. bicalcaratum* have the largest values (Table 3). Furthermore, if tail length is normalized using tarsus length to correct for body size, *P. chalcurum* does not have an unusually long tail compared with the other species (Table 3). Instead, the results of Davison (1985a) may be driven by the short wing length, relative to tarsus, in *P. chalcurum* (Table 3).

Relative to its closest ornamented relative (*P. bicaratum*), *P. chalcurum* does not exhibit a high degree of sexual dimorphism for tail size (Table 2), which might be expected if there were strong sexual selection for tail length in males in *P. chalcurum*. In another study, Davison (1985b) reported a higher correlation between tail and wing length in male *P. chalcurum* than in other *Polyplectron* taxa, though sexual selection on tail length would be expected to decrease rather than increase the correlation between tail length and body size when compared with other species. Thus, there is no evidence that females have shifted attention from ocelli to tails in *P. chalcurum* (or *P. inopinatum*). Although we have examined the likely types of traits which may be used in sexual selection, we may not have identified the appropriate trait, particularly if the shift has been to a completely novel trait such as has occurred in bowerbirds (e.g. Gilliard, 1956; Kusmierski *et al.*, 1993). However, it is difficult to imagine what this novel trait may be.

The reduction of ornamentation in *P. inopinatum* is particularly interesting. Two recent studies have found that sexual selection favoured males with reduced ornaments (Saetre *et al.*, 1997; Griffith, Owens & Burke, 1999). Hybridization avoidance, suggested as one reason females may shift to reduced male ornamentation (Saetre *et al.*, 1997) is possible in *P. inopinatum*, though altitudinal differences separate this species from *P. malacense*. Alternatively, female choice may shift to resources provided by males (e.g. parental care) under some conditions, and these may show a negative relationship with male trait size (Griffith *et al.*, 1999), particularly if males experience trade-offs between ornamentation and other resources important in reproduction.

Interestingly, the two least ornamented species, *P. chalcurum* and *P. inopinatum* inhabit montane regions, whereas other *Polyplectron* species primarily inhabit lowland rain forest. Thus, although there does not appear to be a change in the trait(s) used in sexual selection, as many models predict, it is possible that changes in signal transmission, natural selection or available resources may be driving the observed differences (e.g. Marchetti, 1993; Wiens, 1999). Unfortunately, the small number of taxa in *Polyplectron* makes testing the importance of habitat on the degree of ornamentation impossible. It is also of interest that Badyaev (1997) found altitudinal differences in sexual dimorphism in finches, with males at high altitudes being reduced in brightness relative to species at lower altitudes. These differences could be driven by factors such as resource limitation at higher altitudes or a shorter mating season (see Badyaev, 1997), though the impact of such factors on *Polyplectron* is not known.

THE CLOSEST RELATIVES OF *POLYPECTRON*

We used the large CYB alignment to examine the closest relatives of *Polyplectron*. The ML and single MP tree contained a 'pavonine' clade containing *Polyplectron*, *Pavo*, *Afropavo*, *Argusianus* and *Rheinartia* (Fig. 3), though bootstrap support using equally weighted parsimony was less than 50% for uniting these taxa (Fig. 3). Bootstrap analyses using different parsimony weighting schemes found a monophyletic pavonine clade in all but transversion-only analyses. However, bootstrap support for pavonine monophyly never exceeded 50% in any analysis. Overall, our data suggest the pavonine clade is monophyletic, though additional data will be necessary to demonstrate this conclusively and to determine the branching patterns of the three lineages within the clade if it exists.

The patterns of ornament and display evolution seen in *Polyplectron* are paralleled by the other pavonine species (Table 1). Ocelli are found in male *Argusianus*, where they are restricted to greatly elongated secondaries of the wings, which can be up to a metre in length. In contrast, the closely related *Rheinartia* lacks ocelli. Male *Pavo* have ocelli, while *Afropavo* lacks visible ocelli (although vestigial ocelli may be present; Lowe, 1938). Both *Argusianus* and *Pavo* have frontal displays, in which the ocelli are prominently presented to females (in *Argusianus* the secondaries are spread to form a fan encircling the male's head), while *Rheinartia* exhibits only a lateral display. *Afropavo* is interesting, in that it lacks visible ocelli but performs a frontal display. This indicates that, unlike *Polyplectron*, loss of the morphological trait is not coupled to loss of the display behaviour in this lineage. Crests and bare facial skin are also present in many of the other pavonine species, particularly those with ocelli and frontal displays (Table 1). It is not clear whether the complex ocelli are homologous, though if pavonine birds form a clade (e.g. Fig. 3), it would support homology of ocelli among the pavonines. Alternatively, there may be a homologous, pre-existing sensory bias (e.g. Ryan *et al.*, 1990) for ocelli within this group, such that female choice has selected for ocelli independently in all of the lineages.

CONCLUSIONS

Contrary to earlier hypotheses, a rigorous phylogeny of *Polyplectron* indicates that, within this group, ocelli have been lost rather than gained. Although sexual selection has not been studied in these taxa, the apparent importance of ocelli in displays suggests that, in many taxa, sexual selection for ocelli occurs. This study emphasizes that the focus of sexual selection may change or be lost within a lineage, and that loss of ornaments may be as common as their gain (e.g.

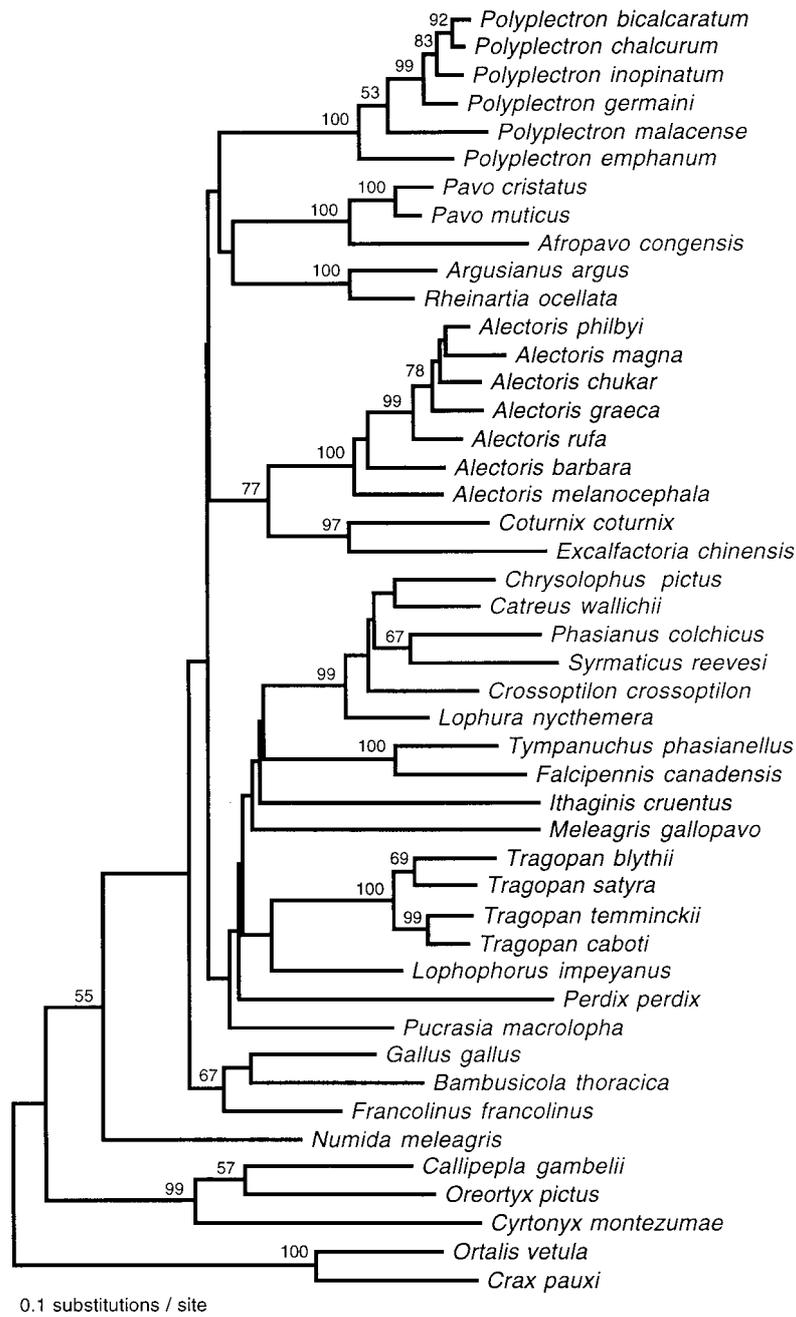


Figure 3. Maximum likelihood phylogeny of the large CYB alignment. Numbers at nodes indicate bootstrap support from equally weighted parsimony analysis. Bootstrap proportions <50% are not shown.

Wiens, 1999). In addition, factors affecting the loss and reduction of male ornamentation needs to be better explored theoretically and empirically. Comparative studies of courtship displays and female choice in genera such as *Polyplectron* may help to elucidate which factors may be important in determining when sexual selection may change, be reduced or lost, and

how these factors may affect the degree of ornamentation in males.

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