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<https://escholarship.org/uc/item/1jv149kh>

Journal

Ibis, 138(1)

ISSN

0019-1019

Author

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Publication Date

1996

DOI

10.1111/j.1474-919x.1996.tb04309.x

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Three fundamental contributions of molecular genetics to avian ecology and evolution

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Studies in molecular genetics are having revisionary impact in at least three broad areas of avian ecology and evolution: mating systems, geographic population structure and gene flow, and phylogenetic relationships among species and higher taxa. With regard to mating systems, genetic analyses of maternity and paternity have revealed unexpectedly high frequencies of extra-pair fertilization and intraspecific brood parasitism in numerous avian species (including those thought to be socially monogamous), and these discoveries are prompting a fundamental reshaping of mating system theory for birds. With regard to genetic structure, molecular markers have uncovered a great variety of depths and patterns in the phylogeographic histories of conspecific populations, and these findings provide novel perspectives on historical gene flow regimes and species concepts. With regard to evolutionary relationships among higher avian taxa, molecular findings have suggested several phylogenetic realignments, thus prompting renewed interest in the cross-comparative aspects of molecular and morphological evolution as well as of alternative procedures for molecular analysis.

The molecular revolution in ecology and evolution, which gained great momentum with the introduction of allozyme methods in the mid-1960s, will soon be entering its fourth decade (Avisé 1994). Especially in recent years, molecular studies on avian species have figured prominently in this literature. Here I will highlight three major areas in avian biology where molecular findings are revolutionizing thought. These research programmes are illuminating because they span a hierarchy of topics ranging from micro-evolutionary to macro-evolutionary, because collectively they involve nearly the entire panoply of available molecular laboratory procedures and also because they exemplify the stimulating interplay between theory and empiricism that can characterize science during periods of active discovery.

MATING SYSTEMS

With respect to observed social interactions, birds traditionally have been considered among the most monogamous of organisms—only about 10% of species were thought to engage regularly in polygynous or other nonmonogamous mating behaviour during a given breeding period (Lack 1968). Indeed, this perception had an important influence on the early development of general theories for animal mating

systems. The following is a simplified synopsis of one conventional line of argumentation.

If the behavioural predispositions underlying mating systems can be viewed as evolved responses to selection pressures favouring the selfish genetic interests of individual males and females (rather than as fully harmonic reproductive ventures), then idealized reproductive strategies are expected to differ between the sexes (Trivers 1972, Emlen & Oring 1977). In general, natural selection should tend to push the genetically based mating proclivities of males towards polygyny (matings with multiple females) and those of females towards polyandry (matings with multiple males, particularly if males could also be enlisted to provide exclusive care for the female's offspring) (Fig. 1). Because these gender-based selective pressures are in opposition, realized mating patterns presumably reflect various compromises arbitrated by other ecological and evolutionary circumstances. Two fundamental evolutionary considerations are sexual asymmetries in (1) overall energetic investment in particular bouts of reproduction, including the costs of gamete production (which are normally much higher for a female; thus, female reproductive success tends to be limited by access to resources whereas male reproductive success tends to be limited by access to females [Davies 1991]) and (2) an adult's confidence of biological parentage (females normally have

Figure 1. One traditional view of animal mating systems, in which opposing reproductive strategies of males v females lead to an evolutionary "tug-of-war" whose resolution in a given species is influenced by additional ecological and evolutionary selection pressures (see text).

greater assuredness of genetic maternity for the progeny they rear than do males of genetic paternity). Presumably for these reasons, polyandrous mating systems in which males assume primary responsibility for care of offspring can evolve only rarely. In practice, the strategic "battle of the sexes" along a polygyny–polyandry continuum (Fig. 1) therefore reduces to an evolutionary tug-of-war between behavioural polygyny and monogamy, with the outcome for a given species influenced by additional factors.

One such factor is whether a male *can* contribute substantively to a couple's reproductive output (beyond gametic donations *per se*). For example, in many herbivorous mammals, males simply are ill equipped for progeny rearing because they lack functional mammary glands and otherwise can furnish little assistance to young. Perhaps these factors tip the evolutionary scale in favour of polygyny in these species (a female's reproductive output would not likely be enhanced by engaging in strict monogamy). On the other hand, most male birds are biologically well equipped to share in parental duties such as nest construction, territorial defence and food acquisition, and perhaps for this reason, monogamy tends to evolve (a female's fitness *can* be enhanced by the added contributions of devoted fathers to progeny rearing). Thus, in the study of avian mating systems, a conceptual challenge as traditionally perceived has been how to account for the occasional instances of known or suspected behavioural polygyny. For example, special ecological circumstances were proposed in which a female's fitness might be enhanced if she became the second or third mate of a polygynous male of high quality (e.g. one defending a deluxe territory or one endowed with good genetic traits) rather than remain the sole mate of a monogamous but otherwise low-quality male (e.g. Verner & Willson 1966, Orians 1969).

Though such scenarios appear reasonable, any theory must be evaluated against empirical experience, and recent discoveries based on molecular assays have fundamentally challenged conventional wisdom about the true nature of

avian mating systems. From molecular analyses of genetic parentage based on protein (allozyme) and DNA restriction-fragment-length-polymorphism markers, definitive evidence has accumulated for widespread cuckoldry in birds (see Westneat *et al.* 1990, Birkhead & Møller 1992, Avise 1994, Westneat & Webster 1994 for reviews of the recent literature). In nearly 75% of about 40 genetically assayed species (involving more than 6000 offspring from c. 1700 broods), at least some progeny have proved to be sired by males other than the apparent caregiver or territory holder that was socially affiliated with the offspring's mother. Reported frequencies of successful "extra-pair fertilization" (EPF) have varied considerably across species but not uncommonly involve more than 15% of total nestlings and 30–40% of all broods. To cite but one example, studies of allozyme profiles and DNA fingerprints in the Indigo Bunting *Passerina cyanea*, a species formerly thought to be predominantly monogamous, revealed that about 35% of total progeny (in nearly 50% of all broods) had been sired by cuckolding males (Westneat 1987, 1990).

Perhaps even more surprising are the growing number of genetic reports where an appreciable fraction of hatchlings carry genotypes inconsistent with transmission from the putative mother. These results apparently reflect the occurrence of intraspecific brood parasitism (IBP) or "egg dumping", wherein females lay eggs in the nests of conspecifics (reviews in Petrie & Møller 1991, Avise 1994, Westneat & Webster 1994). For example, DNA fingerprints of 92 offspring in 25 families of the Zebra Finch *Taeniopygia guttata* revealed that about 10% of offspring and 36% of broods resulted from IBP (Birkhead *et al.* 1990) and a similar study of House Wrens *Troglodytes aedon* based on allozymes suggested that as many as 30% of chicks had biological mothers other than the nest attendant (Price *et al.* 1989).

Not all molecular studies of avian mating systems have uncovered evidence for frequent extra-pair fertilizations (e.g. Gyllenstein *et al.* 1990, Hunter *et al.* 1992, Decker *et al.* 1993, Swatschek *et al.* 1994, Warkentin *et al.* 1994) or intraspecific brood parasitism (e.g. Smyth *et al.* 1993), but nonetheless these phenomena appear far more widespread than formerly supposed. Already, "second-generation" analyses of parentage have begun to appear which go beyond mere documentation of intraspecific brood parasitism and extra-pair fertilization and attempt to couple genetic assessments of maternity and paternity with field studies of avian behaviour and ecology (examples in Table 1). The net result of such molecular analyses is an emerging view in which the reproductive strategies of most avian species appear far more varied and complex than formerly supposed. Genetic documentations of illegitimate young, often in high frequency, suggest that conventional field observations on the social interactions between males and females are, at best, imprecise indicators of actual gene transfer across generations.

Many more studies like those listed in Table 1 are needed before the ecological or evolutionary significance of these new findings can be fully appreciated, but outlines of several new paradigms regarding avian mating systems already are

Table 1. Examples of mating system studies which coupled molecular parentage assessments with behavioural or ecological data from natural avian populations

Species	Reported findings	Reference
Eastern Bluebird <i>Sialia sialis</i>	Frequency of being cuckolded was greater for males that (1) were in their first breeding season; (2) paired with females who tended to stray off territory; (3) exhibited stronger "mate-guarding" behaviour (at face value, a counter-intuitive result)	Gowaty & Bridges 1991
Purple Martin <i>Progne subis</i>	Frequency of successful EPFs was greater for older males	Morton <i>et al.</i> 1990
Red-winged Blackbird <i>Agelaius phoeniceus</i>	Proportions of illegitimate chicks were greater in marshes with higher male densities; cuckolding males often were territorial neighbors	Gibbs <i>et al.</i> 1990
Barn Swallow <i>Hirundo rustica</i>	Males with longer and more symmetrical tail streamers showed increased susceptibility to cuckoldry (but this was offset by a female pairing preference for such males)	Smith <i>et al.</i> 1991
Blue Tit <i>Parus caeruleus</i>	Mate guarding by males was relatively ineffective in protecting paternity	Kempnaers <i>et al.</i> 1992
Striped-backed Wren <i>Campylorhynchus nuchalis</i>	Subordinate male "helpers at the nest", which are young from prior broods in this cooperatively breeding species, themselves sometimes achieved successful fertilizations within the kinship group	Rabenold <i>et al.</i> 1990
Spotted Sandpiper <i>Actitis macularia</i>	In this polyandrous species, early mating males tended to cuckold their female's later mates via sperm storage in the female's reproductive tract	Oring <i>et al.</i> 1992
Swampphen <i>Porphyrio porphyrio</i>	In this polygynandrous species characterized by strong behavioural dominance hierarchies, social rank and copulation frequency were concluded by the authors to be poor predictors of male reproductive success	Lambert <i>et al.</i> 1994
Bobolink <i>Dolichonyx oryzivorus</i>	Younger males and older females were more likely to have EPF young in the nest	Bollinger & Gavin 1991

taking shape. First, the traditional wisdom that reproductive variance among males is much higher in polygynous than in monogamous taxa may require re-examination; EPFs by "subordinate" or "floater" males could appreciably decrease the variance in male reproductive success in socially polygynous populations, and EPFs by cuckolding males could potentially increase this variance in socially monogamous species. If so, conventional arguments that a much greater intensity of both intra- and inter-sexual selection characterizes "polygynous" as compared with "monogamous" species could be weakened considerably. (Indeed, this may contribute to the difficulty ornithologists have experienced in documenting predicted relationships between the degree of sexual dimorphism and the socially defined mating system [e.g. Harvey & Bradbury 1991].) Furthermore, if intermale variances in reproductive success are lower than previously supposed, the magnitude of the disparity from the corresponding interfemale variances in fitness may be less also, with possible selective consequences extending to numerous aspects of gender-specific mating strategies.

Second, because of the unexpectedly high frequency of EPFs in many avian species, increased attention has been paid to the possibility that females actively pursue extra-

pair copulations (rather than acting as passive or coerced recipients of EPF-seeking males, as is assumed under traditional mating system theory) (Gowaty & Bridges 1991). Hypothetically, females could achieve fitness advantages from such EPF-pursuit behaviour, such as fertilization insurance, acquisition of food during courtship feeding, elicitation of greater male care, gain of "better" paternal genes for offspring and production of progeny with a broader diversity of genotypes. Furthermore, with sperm from multiple males sometimes jointly placed within a female's reproductive tract, opportunities arise by which natural selection might foster the evolutionary appearance of male-male intergametic competition mechanisms, as well as agencies for active sperm choice by females (Birkhead & Møller 1992). These possibilities might profitably be further examined experimentally.

Third, if intra-specific brood parasitism proves to be as common as some of the early genetic studies indicate, this will necessitate a fundamental re-evaluation of the conventional wisdom that assurance of maternity for females attending an avian brood is invariably much greater than the corresponding assurance of paternity for male caregivers. Although fertilization in birds is internal, most embryonic development takes place outside the female's body, thus

Table 2. Comparative magnitudes of conspecific population structure, as assayed by multilocus protein electrophoresis, for species in each of five vertebrate classes

Taxonomic group	Barrowclough (1983)		Ward <i>et al.</i> (1992)	
	Interpopulation differences ^a ($\pm$ s.e.)	No. of species	Interpopulation differences ^a ($\pm$ s.e.)	No. of species
Birds	0.022 $\pm$ 0.005	5	0.076 $\pm$ 0.020	16
Fishes	0.114 $\pm$ 0.046	9	0.135 $\pm$ 0.021	79
Mammals	0.230 $\pm$ 0.037	25	0.242 $\pm$ 0.030	57
Reptiles	0.304 $\pm$ 0.103	3	0.258 $\pm$ 0.050	22
Amphibians	0.383 $\pm$ 0.073	15	0.315 $\pm$ 0.040	33
Total		57		207

^a Estimates of the among-population component of genetic variance, as estimated by Wright's (1978) F_{st} measure (Barrowclough 1983) or Nei's (1973) G_{st} statistic (Ward *et al.* 1992).

opening an evolutionary window of opportunity for maternal foster-parentage that now appears to have been realized in at least some species via IBP. In the absence of complete maternity assurance, the reproductive options and potential strategies for females become much more varied than formerly supposed, leading to the possibility of co-evolutionary "games" between brood parasites and their conspecific hosts.

Finally, IBP and EPF make virtually inevitable even stronger potential conflicts of interest between parental caregivers. Furthermore, theory suggests that cuckoldry should influence the evolution of parental investment strategies in offspring (Owens 1993, Westneat & Sherman 1993). For example, a caregiver might logically be expected to reduce efforts on behalf of nestlings if there is an increased probability that the young had been sired or dammed by other individuals. This raises the spectre of the evolution of refined capabilities for kin recognition and nepotism by caregivers stemming from the enhanced selection for within-group kinship discrimination (e.g. between full- and half-sibs) that might be anticipated under mating systems that include regular IBP or EPF. In general, because EPF and IBP will tend to reduce the mean coefficients of genetic relatedness between adults and associated young, as well as among nest-mates, these phenomena may be expected to produce selection pressures influencing the evolution of genetically based behavioural predispositions underlying virtually any parent-parent, parent-offspring or offspring-offspring interactions.

POPULATION STRUCTURE AND GENE FLOW

Avian populations have long presented an enigma with regard to postulated levels of geographic structure and magnitudes of gene flow. On the one hand, most birds have a potential for exceptional vagility, given their capacity for

flight and frequent tendency for migrational behaviour. Indeed, numerous studies based on allozyme frequencies suggest that avian populations tend to exhibit less geographic structure (and higher inferred rates of gene flow) than do conspecific populations of many other vertebrates such as freshwater fishes, small mammals, reptiles and amphibians (Table 2). On the other hand, many birds exhibit strong fidelity to natal sites for reproduction, thus suggesting severe restrictions on contemporary genetic exchange between populations. Furthermore, conspecific populations often show obvious geographic variation in such features as song, body size or plumage.

In recent years, analyses of the rapidly evolving mitochondrial DNA (mtDNA) molecule have inspired a novel class of genealogical perspectives on population structure and gene flow in birds. The phylogenetic nature of information content that is inherent in mtDNA (due to its non-recombining, maternal inheritance) has fostered a growing appreciation of the relevance of historical demographic factors to the "phylogeographic" distributions of lineages in contemporary populations. Several lengthy reviews have summarized empirical findings and concepts on mtDNA phylogeographic patterns in various animal taxa (Wilson *et al.* 1985, Avise *et al.* 1987, Avise 1989), including birds (Avise & Ball 1991). Here, space permits only a brief reiteration of some salient points.

Empirically, several avian species have displayed little or no detectable geographic structure in mtDNA lineages across broad areas such as the North American continent. Examples include the Red-winged Blackbird *Agelaius phoeniceus* (Ball *et al.* 1988), Downy Woodpecker *Picoides pubescens* (Ball & Avise 1992) and Chipping Sparrow *Spizella passerina* (Zink & Dittmann 1993b). On the other hand, regional population assemblages within several avian species have proved to exhibit remarkably "deep" separations in mtDNA genealogy, thus strongly suggesting severe interregional restrictions on gene flow. For example, in both the Sharp-tailed and Seaside Sparrows *Ammodramus caudacutus* and *Ammodramus maritimus*, deep and geographically oriented mtDNA phylogenetic "breaks" cleanly distinguish birds from different portions of the respective species' ranges (Fig. 2). Nonetheless, the observation of a statistically significant phylogenetic break in mtDNA genealogy is not alone sufficient to guarantee an absence of contemporary gene flow at the population level, as can be demonstrated clearly by reference to genetic findings in the Snow Goose *Anser caerulescens*. In that species, two distinct mtDNA clades also were observed (Fig. 2), but unlike the situation in the sparrows, these lineages co-occurred within all surveyed populations (which by independent evidence were known to interbreed freely). At least two possibilities exist. Perhaps the divergence between distinctive mtDNA clades in Snow Geese was initiated during earlier (e.g. Pleistocene) population separations, with subsequent dispersal and gene flow leading to the contemporary mtDNA distribution. Or perhaps the genealogical separation represents retention of an ancient mtDNA lineage polymorphism within one or more populations that were in contin-

Figure 2. Frequency histograms of mtDNA genetic distances observed within each of three avian species (after Avise *et al.* 1992).

uous contact. In either event, this example highlights an important distinction that must be drawn between the genealogy of a locus (the phylogenetic history of any piece of DNA, nuclear or mitochondrial) and the composite population pedigree, of which any gene genealogy represents only a tiny sample of realized genetic transmission pathways.

Under what circumstances, then, might lineage distributions within particular "gene trees" (such as those of mtDNA or of any randomly chosen nuclear gene) be informative in studies of population structure? As elaborated elsewhere (Avise 1994), significant discontinuities in a gene genealogy most likely evidence long-term historical separations at the population level when they (1) cleanly and consistently distinguish individuals from different geographic regions (especially when these regions constitute separate biogeographic provinces or subprovinces as identified by indepen-

Figure 3. Schematic representation of the phylogeographic distributions of gene-tree branches for a species whose local populations exhibit an isolation-by-distance effect superimposed over a long-term vicariant separation between regions. Vectors (arrows) in the present-day populations (top) represent contemporary dispersal of organisms, which is assumed to be far less per generation than the total geographic range occupied. Note that extant lineages in the more inclusive or older clades (e.g. time levels a, b and c) tend to show broader geographic distributions in contemporary time than do extant lineages in less inclusive, younger clades. The nodes in the diagram can be interpreted either as reflecting the true genealogy at a locus or as signifying *de novo* mutations by which particular clades were identified.

dent evidence), (2) show geographic patterns that are concordant with those of other genetic traits (as in the Sharp-tailed Sparrow [Greenlaw 1993, Rising & Avise 1993] and in the Fox Sparrow *Passerella iliaca* [Zink 1994]) or (3) show phylogeographic patterns that are concordant with those of other co-distributed species similarly assayed (as in the Seaside Sparrow [Avise 1992]).

Of course, historical vicariant separations are not the only forces that can produce significant population genetic structure, even under scenarios of strict neutrality for genetic markers. Consider a continuously distributed species in which geographic populations are "isolated by distance", i.e. in which the single-generation dispersal distances of individuals or their gametes are far lower than is the total distributional range of the species (Fig. 3). Inevitably, new mutations (derived genetic states) arise at various loci, and some of these will survive to become spatially dispersed as a historical function of the reproductive success and vagilities of the individuals through which they are passed. Through time, superimposed mutations may also arise at some of these genes, thus creating a recognizable hierarchy of increasingly deep clades in a genealogical reconstruction based on these mutations (Fig. 3). However, due to the idiosyncratic nature of mutational origins (as well as to the vagaries of allelic transmission under Mendelian inheritance), the particular arrays of individuals or demes recognizable at any horizon in time will almost certainly differ from one genetic

locus to the next. Thus, although generalized restrictions on gene flow within the species may be implicated by localized geographic distributions of genetic attributes, the particular "populations" recognizable by any single locus will usually be of no special phylogenetic consequence. Furthermore, different suites of genes (and the organismal characters influenced, e.g. plumage features, body size and song dialects) may differ considerably in rate of evolution, such that different assays could reveal distinct patterns of geographic variation. For example, the Song Sparrow *Melospiza melodia* displays a relative paucity of geographic structure in mtDNA haplotypes across North America despite marked geographic variation in size and plumage colouration. These observations were interpreted by Zink and Dittmann (1993a) to be evidence of postglacial dispersal which distributed mtDNA haplotypes continent-wide, followed by rapid *in situ* differentiation in morphological features.

Recently, a multigeneration random-walk model was developed and applied to empirical data on mtDNA lineage distributions in several animal species whose populations were suspected of isolation by distance (Neigel *et al.* 1991, Neigel & Avise 1993). Unlike most previous methods, the approach incorporates the process of dispersion of mutations (rather than assuming an equilibrium between genetic drift and gene flow) and yields estimates of single-generation dispersal distance (rather than magnitudes of gene flow) consistent with observed geographic distributions of mtDNA lineages of various evolutionary age. One avian species included in the analysis was the Red-winged Blackbird, which previously had been shown to exhibit only "shallow" mtDNA phylogeographic structure across North America (Ball *et al.* 1988). When interpreted against the model, estimates of standard dispersal distances were about 3–4 km per generation or roughly 30-fold higher than comparable estimates for three rodent species similarly analysed. Furthermore, the geographic distribution of mtDNA lineages in the Red-winged Blackbird suggested that an equilibrium between migration and genetic drift had been achieved such that older and younger mtDNA clades had similar variances in geographic position. By these same criteria, the rodent species (whose dispersal presumably is more limited) exhibited non-equilibrium population structures in which older clades had significantly greater variances in geographic position than did younger clades.

Another topic in ornithology where such genealogical perspectives on population structure have become relevant involves species concepts. Recently, several authors have proposed that the traditional "biological species concept" (which, ironically, was largely motivated by early studies on birds and was thought to apply especially well to avian taxa [e.g. Mayr 1963]) lacks a sufficient phylogenetic perspective and should be replaced by a "phylogenetic species concept" (PSC). Under the proposed PSC, a species is defined as a monophyletic group composed of "the smallest diagnosable cluster of individual organisms within which there is a parental pattern of ancestry and descent" (Cracraft 1983). Some early formulations of the PSC suggested that "reproductive iso-

lation should not be a part of species concepts" and that even a single genetic trait, no matter how "trivial", might be sufficient for defining a clade and hence a phylogenetic species (McKittrick & Zink 1988). It is with the nature of evidence required for phylogenetic distinctions, and not with the general importance of phylogenetic considerations, that I take issue. For a much extended treatment of what follows, see Avise and Ball (1990).

For the reasons illustrated above, no single gene genealogy (nor phylogenetic distribution of a single genetic trait) should automatically be conflated with an entire organismal phylogeny or pedigree. Normally, it is only when concordant partitions are observed in the distributions of multiple genetic attributes (ideally, those encoded by unlinked and non-epistatic loci) that important historical separations at the populational or species level may justifiably be inferred (see also Kluge 1990). One factor expected to generate such concordance is intrinsic reproductive isolation (the focal point of the biological species concept). Biological species isolated for sufficient periods of time (relative to effective population size) almost inevitably diverge such that numerous genetic traits will come to distinguish them in concordant fashion. Indeed, in traditional systematic practice, the concordant grouping of operational taxonomic units by multiple traits serves as the usual surrogate for direct knowledge about reproductive isolation *per se*, which seldom can be assessed directly in nature (Sokal & Crovello 1970). However, genealogically concordant differences also may arise between populations via longstanding but purely extrinsic (i.e. geographic) barriers to gene flow. In these latter cases, long-term evolutionary persistence of the phylogenetic partitions remains in jeopardy (because the operational taxonomic units might later rejoin and interbreed). Nevertheless, such phylogenetic partitions in organismal pedigree, as recognized by concordant partitions in multiple independent traits, should demarcate populational units worthy of special taxonomic recognition. (Of course, in practice, the question of precisely "how much" concordance is required for taxonomic recognition may remain contentious in particular instances, but in my view this is inevitable given that "speciation" and "subspeciation" tend to be gradual evolutionary processes [especially in outcrossing organisms such as birds] and that magnitudes of genetic exchange and historical "connectedness" can in principle vary continuously along a spectrum of spatial and temporal scales.)

In any event, from such considerations, Avise and Ball (1990) suggest how principles of genealogical concordance might usefully be added to both biological and phylogenetic species concepts. They urge a retention of the philosophical stance of the biological species concept in species recognition, in no small part because intrinsic reproductive isolating barriers will tend through time to forge concordant phylogenetic partitions at multiple loci throughout the genome. Populations separated by long-term but purely extrinsic reproductive barriers should be formally recognized also, but as taxonomic "subspecies" reflecting the concordant and phylogenetically derived differences they exhibit in multiple

Table 3. Examples of surprising or controversial conclusions in higher avian systematics reached by Sibley and Ahlquist (1990) from DNA hybridization studies. Each case shown has been re-examined by the other molecular approaches, with general results indicated

Conclusion from Sibley and Ahlquist based on DNA–DNA hybridization	Molecular re-appraisal		
	Assay	Agreement with Sibley and Ahlquist conclusions? ^a	Reference
New World quail highly distinct from Old World phasianids	mtDNA sequences	Yes	Kornegay <i>et al.</i> 1993, Avise <i>et al.</i> 1994
Hoatzin closely allied to Cuculiformes	mtDNA sequences	No or equivocal	Avise <i>et al.</i> 1994
New World barbets related more closely to toucans than to Old World barbets	mtDNA sequences	Yes	Lanyon & Hall 1994
Birds of paradise related more closely to corvines than to bowerbirds	mtDNA sequences	Yes	Helm-Bychowski & Cracraft 1993
New World and Old World vultures polyphyletic	mtDNA sequences	Yes	Hedges & Sibley 1994
Australian passerines a monophyletic assemblage	Microcomplement fixation	Yes, but details disagree	Baverstock <i>et al.</i> 1991
“Pelecaniform” birds polyphyletic	mtDNA sequences	Yes	Hedges & Sibley 1994
Divers and grebes not closely related	mtDNA sequences	Yes	Hedges & Sibley 1994

^a Original papers should be consulted for details and for many important qualifications to the general conclusion stated here.

genetically encoded characteristics. Overall, by adopting these or related concordance guidelines, an important phylogenetic perspective can be added to the biological species concept, and a recognition of the singularly important role of reproductive isolation in achieving phylogenetic partitions at the organismal level will be added to the phylogenetic species concept.

One remaining complication arises when distinct forms which hybridize in nature are not one another's closest relatives (i.e. are non-sister taxa). Such situations may materialize rather frequently because, as emphasized by proponents of the phylogenetic species concept, reproductive compatibility is a “primitive” rather than derived condition whose retention cannot delineate a clade under strict Hennigian phylogenetic principles. Such hybridizing taxa might eventually merge into one panmictic gene pool, or, at the other extreme, only sporadic F_1 hybrids might be produced. Intermediate levels of hybridization and introgression could be envisioned as well. These “grey” situations do not lend themselves easily to classificatory schemes requiring synopsis into “black-and-white” binomials or trinomials (be they based on either the biological or phylogenetic species concepts). In general, reticulate evolution cannot be handled well by any classification procedure that assumes a nested hierarchy of organismal relationships. In truth, much of the current debate about species concepts may stem from a failure to recognize the epistemological impossibility of adequately “squeezing” complex biological situations into simple Linnaean taxonomic summaries. Thus, much more may often be known about both the reproductive relationships and phylogenies of related forms than can be captured in the condensed taxonomic assignments.

SPECIES PHYLOGENIES AND HIGHER SYSTEMATICS

A third arena in which molecular approaches are having significant impact in avian biology and evolution involves assessment of higher systematic relationships (Sheldon & Bledsoe 1993). Especially noteworthy has been a long series of studies based on the DNA–DNA hybridization technique (reviewed in Sibley & Ahlquist 1990). This method involves gauging the magnitude of genetic divergence between genomes from the thermal stabilities of artificially produced heteroduplex DNA molecules, the two strands of which come from species A and B. Thermal stability profiles for heteroduplex molecules are compared with those of intraspecific homoduplexes, and results are summarized as numerical “distance” values which represent estimates of the overall magnitude of genetic divergence between the “single-copy” portions of the genomes of the species being compared. It is especially appropriate to focus on the DNA hybridization approach in the context of avian systematics because no other molecular method has been applied to as many avian taxa (more than 1700 species representing 168 of 171 taxonomic families were included in the Sibley and Ahlquist studies), no other organismal group has been assayed nearly as extensively by this method and no other molecular study on birds has received so much attention or generated so much controversy (e.g. Sarich *et al.* 1989, Diamond 1991, Gill & Sheldon 1991, Cracraft 1992, Lanyon 1992, Mindell 1992).

According to Sibley (1994), most (75%) of their published conclusions from the DNA hybridization approach agree well with traditional views about avian relationships and clas-

sification. The remaining cases are of special interest. A few of these contentious or unexpected conclusions are presented in Table 3, and more extensive treatments can be found in Sibley and Ahlquist (1990) and Sheldon and Bledsoe (1993). For example, one surprising suggestion from DNA hybridization was that the New World quail are highly distinct from Old World quail and allies (Phasianidae) and, indeed, fall outside a clade composed of these Old World game birds plus guineafowl (Numididae).

An important task now before ornithologists is to re-examine some of these controversial suggestions using other kinds of molecular (and nonmolecular) data. One such molecular approach involves direct nucleotide sequencing of slowly evolving genes, and case studies are beginning to accumulate (Table 3). For example, phylogenetic analyses of available cytochrome *b* sequences from the mitochondrial genome appear to support the genetic distinctiveness of the New World quail from the Old World game birds (Kornegay *et al.*, 1993), as does a suggestion based on DNA hybridization that the birds of paradise are not related closely to the bowerbirds (Helm-Bychowski & Cracraft 1993). Mitochondrial DNA sequence data also have been interpreted to support a polyphyletic origin for the totipalmate "pelecaniform" birds (Hedges & Sibley 1994), for New World and Old World vultures (Hedges & Sibley 1994) and for divers and grebes (Hedges & Sibley 1994). Each of these conclusions contradicts conventional wisdom but was suggested from earlier DNA-DNA hybridization evidence (Table 3). On the other hand, cytochrome *b* sequence analyses failed to support an especially close association between the Hoatzin *Opisthocomus hoazin* and cuckoos (Cuculiformes) that had been suggested from DNA hybridization (Avice *et al.* 1994).

Many more tests of this sort will be required before final conclusions are drawn about phylogenetic relationships among higher avian taxa, but regardless of particular outcomes, the results and the controversies themselves have proved illuminating with regard to alternative systematic approaches. In at least two important respects, DNA-DNA hybridization and direct nucleotide sequencing fall near opposite poles (operational and conceptual) in molecular systematics: the former generates quantitative distance values only, whereas the latter reveals qualitative character states potentially suitable for cladistic or parsimony treatments (in addition to distance-based analyses); and the former ideally provides a composite summary of information from multitudinous genes, whereas the latter as normally applied reveals information from a much smaller number of linked nucleotide positions. Additional distinctions between these approaches are detailed in Mindell (1992).

Direct sequence re-appraisals of avian relationships motivated in part by DNA hybridization studies already have yielded several conclusions of broader relevance to the field of molecular evolution. One revelation is that DNA sequences (at least from single genes) seldom provide the phylogenetic panaceas that naively were predicted even a few years ago. At the very least, several complicating factors in phylogeny reconstruction based on single loci have come into

sharper focus. These include (1) evolutionary rate heterogeneities across lineages, (2) extreme rate heterogeneities and variable saturation effects across nucleotide positions (e.g. first, second and third positions of codons), (3) marked differences in the occurrence of alternative classes of character-state change (e.g. transversions v transitions), (4) the possibility of compensatory mutational changes in a stretch of linked nucleotide sites, (5) extensive homoplasy at the level of individual nucleotide characters, (6) plateau or ceiling effects on magnitudes of acceptable genetic change, particularly at greater periods of evolutionary separation, (7) extremely shallow slopes in some of the regressions relating sequence divergence to time (even in the absence of pronounced ceiling effects *per se*), such that only small numbers of sequence changes at a particular gene are expected to have arisen between closely spaced but ancient evolutionary nodes and (8) considerable intraspecific sequence diversities in most species, such that in principle the topology of a particular gene genealogy may truly differ from a composite organismal phylogeny because of the idiosyncratic sorting of polymorphic ancestral allelic lineages across evolutionary nodes that are temporally close (see above and Avice 1994).

It is beyond the scope of this review to further explicate these factors, but in any event a current take-home message is that neither the DNA-DNA hybridization method nor direct nucleotide sequencing of particular genes can automatically be assumed to provide absolute phylogenetic knowledge against which conclusively to appraise alternative phylogenetic methods. In the absence of a definitive procedure for phylogenetic "ground truthing", it appears that systematists can only hope to struggle towards a more reliable phylogeny by accumulating additional information and searching for congruent patterns in the data likely to be indicative of phylogenetic signal.

Robert Zink and Charles Sibley provided useful comments on the manuscript. Work in the Avice laboratory has been supported by grants from the National Science Foundation.

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