

must, however, caution investigators that the generality of our findings is unknown. Metabolic enzymes other than avian CS and HOAD may be affected by time to freezing values of less than 60 min. Therefore, we recommend that tissues be frozen as soon as possible after dissection and that they be kept as cold as possible until they are frozen. Investigators in the field can place tissues in cryogenic tubes and then immerse the tubes into liquid nitrogen for transport back to the laboratory. Should liquid nitrogen be unavailable, tissue samples can be frozen in foil on dry ice.

According to Srere (1969), more CS can be extracted from frozen than fresh tissue, presumably due to the cell lysing that results from freezing. Since both CS and HOAD are mitochondrial enzymes, we expected the activity of both enzymes to be higher in samples that had been frozen. Although the activity of both enzymes was higher in frozen than fresh tissue, the differences were not significant for either CS or HOAD. Consequently, we believe that the sonication of the muscle homogenate involved in the enzyme-activity determination protocol results in thorough cell lysing and that freezing the muscle samples does not significantly enhance the cell-lysing process.

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DNA Fingerprinting in Avian Behavioral Ecology: Two Cultures Arise

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As each of us learns more and more about less and less, it seems inevitable that students of avian mating systems will occupy two cultures. Like C. P. Snow's two cultures—sciences and liberal arts—we are de-

veloping different languages, different methods, and seemingly different questions. The two cultures that now study avian mating systems are represented by those who are importing molecular-genetic tech-

niques to the study of social behavior and those who use the classical approach of estimating reproductive success using behavioral data alone. At times, the first group seems so enamored of techniques that the questions they seek to address seem secondary, whereas the second group remains so focused on their questions and dismissive of technical advances that they resist the promise of the new. At their best, molecular-genetic techniques are revolutionizing the study of mating systems by providing measures of individual reproductive success with accuracy that is unprecedented; at worst, they are fueling an expensive bandwagon that threatens to severely diminish the intellectual and material resources available for traditional studies in behavioral ecology. Others have noted some of these differences between "field workers" and "lab rats" (Pemberton 1991), and it was our observation based on presentations and discussions at a recent meeting (22nd International Ethological Conference [IEC] in Kyoto, Japan 22-29 August 1991) that these cultural divisions among us are widening rather than narrowing. To summarize recent progress in the rapidly developing field of genetic study of avian mating systems, we briefly review presentations at the IEC related to avian mating systems. We then use this material as a basis for remarks on our perceptions of the causes and our prescriptions for a lessening of the gap that threatens this counterproductive budding into two truly distinct cultures.

Four speakers in the symposium "Molecular Genetics and Social Behavior of Birds" presented data using molecular genetic techniques to study questions about kinship in lek-breeding Ruffs (*Philomachus pugnax*) and Black Grouse (*Dendragapus obscurus*), cooperatively breeding Stripe-backed Wrens (*Campylorhynchus nuchalis*), socially monogamous Eastern Bluebirds (*Sialia sialis*), and socially polygynous Red-winged Blackbirds (*Agelaius phoeniceus*). In addition, several authors with contributed papers at the conference presented results of studies also using genetic analyses on other bird species. The molecular and statistical techniques used by the speakers demonstrated the breadth of approaches now available to students of social behavior. Multilocus DNA "fingerprinting" was the most common technique among all the studies, but P. Gowaty (Clemson) showed how the use of allozymes as genetic markers combined with appropriate statistical analyses may still offer answers to some questions by providing an example in birds where actual paternity was related to the amount of paternal care provided. Use of multilocus DNA fingerprints allowed P. Rabenold (Purdue) to demonstrate that subordinate males can occasionally obtain some success in cooperatively breeding Stripe-backed Wrens. In addition, the power of hypervariable single-locus DNA markers for parentage assignments was illustrated in two studies. T. Burke (Leicester) showed how species specific single-locus VNTR probes could be used for assignments of male parents in

lekking species such as Black Grouse and Ruff, where there is no behavioral association between males and females after fertilization occurs. L. Gibbs (McMaster) used MHC-derived single-locus probes to show that male Red-winged Blackbirds obtain an average of 20% of their overall reproductive success from extrapair fertilizations.

In our view, a major conclusion to be drawn from these studies and the others presented was that, at present, no generalizations can be drawn regarding the link between "sociographic" (behavioral) and "genetic" mating systems. In some cases (e.g. bluebirds, blackbirds, and wrens) there are substantial differences between these two ways of defining "mating systems," while in others (*Phylloscopus* warblers and Dunnocks, *Prunnella modularis*) sociographic and genetic mating systems matched, at least in some places at some times. To us, this indicates the absolute necessity of using genetic techniques in studies of reproductive success. This is because for individual species it is still important to determine if behavior is an accurate predictor of reproductive success and, as yet, there are no simple conclusions available about the congruence between conventionally defined mating systems and levels of extrapair parentage.

A troubling observation to us was the lack of critical challenges to the molecular and statistical techniques used for analyzing pedigree information. It seems clear that there were few in our audience who have yet digested the intricacies of the DNA-based markers to the point where they can offer constructive challenges to the technical aspects of the work being reported. The same is true for the subtleties of statistical approaches applied to single-locus systems (e.g. allozymes and single-locus DNA markers) and more complex multilocus systems. This seems to be one important contribution to the incipient development of the two cultures, but one that can be overcome through attention to debates in human forensics (the original field of application of multilocus DNA fingerprinting, where refinements of techniques and methods of analysis are occurring at a rapid pace; e.g. Jeffreys et al. 1991) and elsewhere (Lynch 1988). Many of these refinements can be directly applied to parentage work on birds, and many of the debates have direct applicability to other aspects of our work, such as the makeup of our sample populations (Lewontin and Hartl 1991).

In the round-table discussion that followed the symposium (entitled "DNA Technology in Behavioral Studies"), issues ranged from the technical to the practical. Rauno Alatalo's (Jyväskylä) comments represented a reaction to the developing hegemony of molecular approaches to a field once dominated by field-oriented behavioral biologists. He noted that "we shouldn't be aimlessly throwing these new techniques at our questions," and that compared to our old approaches the molecular approaches are expensive. He lamented that it now seems more important

in our grant applications to say we are using these molecular tools than it is to justify the significance of our questions. He lobbied for careful thought about the application of these methods. He also remarked that, if possible, studies should also incorporate experimental approaches to maximize the yield of information from such analyses. His remarks were buoying to us in that they were informed, critical remarks about the limits to our recent knowledge from DNA fingerprinting studies.

Though at times it may seem that we are in danger of having the tail wag the dog, it also seems to us that techniques still remain secondary to the questions in our field. Part of the reason for focus on techniques is because of their newness in terms of how they are used and what they reveal. There is still considerable debate about what types of probes are most valuable and how to analyze the genetic markers they detect (e.g. Weatherhead and Montgomerie 1991, Pemberton et al. 1991). We see this as a transitory stage that will end, as happened during the development of electrophoresis two decades ago, with the establishment of standard methods for the isolation and use of DNA-based markers, and standard procedures for analyzing the data. Note, however, that there is no reason to expect this developmental stage to go any more quickly than the statistical and analytical stage did for allozymes, with novel statistical approaches arising relatively late (Westneat et al. 1987, Gowaty and Bridges 1991). Another reason for the focus on the techniques rather than on formal hypothesis testing is the novelty of the information on the natural history of mating systems that is being revealed. The results so far suggest that much baseline data on, say, the natural history of extrapair parentage in birds with different sociographic mating systems still need to be collected before we can identify species in which it makes sense to study the adaptive significance of multiple mating by females and males. In species where such information is already available (e.g. Eastern Bluebirds, Red-winged Blackbirds, and Indigo Buntings, *Passerina cyanea*), it is reasonable to start to ask more sophisticated questions about the reproductive behavior of males and females. However, few species have yet been studied in much detail and, therefore, we see the initial application of DNA fingerprinting to a wide variety of species as a useful first step to correcting the void in our natural-history knowledge.

Pemberton (1991) called multilocus DNA fingerprinting clumsy; this evaluation was not absent from the symposium or the roundtable. The difficulties—from reading and interpreting the autoradiographs to statistical interpretation of results—have led some to argue for increased use of single-locus probes, which are far easier to score and whose genetic characteristics are easier to quantify. The major drawback to using such probes is that they seem to be taxon specific. Consistent with this, T. Burke described some

of the technical steps involved in developing systems for the more rapid isolation of such markers and their application to a variety of avian species. Likewise, one of us (P.A.G.) offered an analysis of the quantitative costs and benefits of different molecular approaches given the questions being asked. The consensus seems to be that for studies requiring only rejection of maternity or paternity, multilocus DNA fingerprints work well. However, when assignments of parentage are needed and not just exclusions, multilocus DNA fingerprints are difficult to use except under rare conditions when the number of candidate assignees is small. For such studies, single-locus markers seem far preferable because they are far easier to score, characterize genetically, and evaluate statistically.

Another issue raised during the round-table discussion was the reliability and generality of conclusions. What do we conclude and how do we proceed? Once we have a description of the concordance or lack thereof between measures of sociographic success and genetic success, does the need for additional testing at the genetic level remain? Clearly, that is a question whose answer depends on our confidence in our methods, and the potential for biologically interesting variation across populations. Given that the sociographically measured reproductive success of polygynous Red-winged Blackbird males may correlate with genetic success in one study (D. Westneat 1991, 109th AOU Meeting, Montreal), but not in another (Gibbs et al. 1991), it seems premature to stop using genetic techniques to investigate social behavior in this species at least. However, will the skeptics be put to rest if no evidence of extrapair paternity is found in Florida Scrub Jays (*Aphelocoma coerulescens coerulescens*), even if duplicate blood samples are studied in independent laboratories?

Some behavioral ecologists seem to feel that once reproductive success can be described in genetic terms then answers to our many questions about how selection acts on variation will immediately become clear. No doubt, some of our questions will be answered. But, as a generality, we feel this is too optimistic a view. Evolutionary biologists have been attempting to measure selection in the wild for a long time using components of fitness, such as survival, and one lesson from these attempts (cf. Endler 1986) is that there is usually a large random component to variation in fitness among individuals, no matter how accurately either the trait or component of fitness is measured. Thus, even with the accurate estimates of reproductive success based on DNA methodologies, large samples and possibly experiments will still be required to make sense of the variation in reproductive behavior in females and males. We hope that appreciation of this and other limits to DNA technologies will come if the community at large learns the intricacies and details of the methods, so that those of us in the trenches can receive the construc-

tive challenges and criticism that are essential to real advance. We are on the cusp of integration; what we need more than ever at this point is meaningful dialogue between workers in the two cultures.

A major issue facing the community at large is the potential impact of costs of molecular-based analyses on funding for field studies of behavior in general. A question is, in these times of limited budgets, will the increased demand for expensive molecular analyses reduce the amount of funding for equally valuable but less trendy work that uses only behavioral techniques? Judging from what has happened in the field of systematics, which also experienced a molecular revolution starting about 10 years ago, the answer likely will be yes. An informal perusal of public information available on the grants awarded by the National Science Foundation (USA) Systematics Panel suggests that over this period the increased use of molecular techniques has resulted in an increase in the cost per grant and, as a consequence of no net increase in overall funding, a decline in the total number of grants awarded (W. S. Moore pers. comm.). Discussion has focused on two recent suggestions (cf. Weatherhead and Montgomerie 1991) as to how behavioral ecologists should respond to this impending situation: (1) by making a concerted effort to convince national funding agencies that the costs of doing research in this field have changed fundamentally and more money is needed; and (2) by establishing national centers that will carry out such analyses on a fee-for-service basis, so that the limited resources now available for this work may be more economically used. The second option seems a possibility for straight-forward applications (parentage exclusions) using standardized methodologies (multilocus DNA fingerprinting). However, we agree with Pemberton et al. (1991) that much additional developmental work needs to be done in this very young area of behavioral ecology. We also feel such work is better carried out through collaborations on an individual basis between laboratory and field workers in situations that foster the development of novel genetic and statistical applications.

Obviously, the best way to discourage further fractionating into two cultures is for more of us to embrace the painful process of mastering the details of both. A possibly more reasonable alternative is close collaboration between field and laboratory biologists. Forging close, cooperative, and productive collaborations is not easy—in our experience they take considerable time and effort to develop, because of the frequent misunderstandings and false expectations that characterize the early stages of such relationships. However, once operating smoothly they can be immensely productive. Such collaborations between workers in disparate fields are a tradition in other areas of sciences; we feel strongly that now is the time for behavioral ecology to develop such a tradition. If researchers choose to collaborate, we rec-

ommend that the laboratory biologists visit the field often, and that the field biologists visit the laboratory equally often. We think these close collaborations can be especially productive when the laboratory biologist understands that the field work is complicated and can be expensive. And, although field work is almost always fun, it requires time, skill, and ingenuity, and involves more than catching a bird and collecting a tube of blood. Likewise, field biologists should realize that the production of DNA markers is a complicated, expensive, and sometimes frustrating process that also requires considerable time, skill, and ingenuity. We also recommend that the questions and not just the answers be forged in collaboration. These may be the most significant and simplest ways to minimize the emerging gap between our two cultures.

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