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Received 27 September 1988, accepted 28 September 1988.

The Study of Geographic Variation

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The study of geographic variation has occupied a prominent place in ornithology. Historically most students of geographic variation were motivated by taxonomic interests, primarily subspecies description. Recent analyses of geographic variation have considered various topics of evolutionary interest. Two assumptions of most evolutionary studies of geographic variation are that geographic differences represent local adaptation, and that geographic differentiation is a stage in the speciation process. How these topics are studied is not universally agreed upon. Rising's (1988a) review of my Fox Sparrow (*Passerella iliaca*) study (Zink 1986) permits consideration of contemporary approaches to the study and interpretation of geographic variation.

Adaptation.—My results suggested high gene flow and near genetic identity of 31 "populations" at 38 protein-coding loci. This does not assure genetic uniformity at other loci. It does suggest a panmictic population structure, whereas inferences from morphological patterns might indicate a highly substructured population. Furthermore, available evidence (summarized in Barrowclough et al. 1985) suggests that enzyme variation is consistent with predictions of the mutation-genetic drift model of neutral theory and therefore useful for estimating gene flow and population histories. Thus, observed enzyme variation appears nonadaptive, although this does not rule out selection at other loci. From the observations of high gene flow and genetic uniformity, and a lack of environmental-morphometric associations, I suggested that the degree of morphological differentiation might

not be a reliable guide to genetic differentiation even at loci influencing morphology; environmental induction was posed as a potential mechanism of morphological divergence. Without the genetic data I probably would have favored the traditional interpretation that adaptation (e.g. natural selection) must be responsible for geographic differences in morphometrics but I simply could not find the telling environmental correlations (see Gould and Lewontin 1979).

My conclusion that geographic patterns of morphological variation were not necessarily results of adaptation prompted Rising to conjure an image of neutralistic "euphoria." This caricature denigrates an important body of empirical and theoretical knowledge on the evolution of genes in populations. For too long we have accepted uncritically the assertion (which might be true) that adaptation alone causes geographic differentiation, without tests of alternatives (see Lande 1985, Lynch and Hill 1986). For example, Rising advocated analysis of Fox Sparrow (*Passerella iliaca*) foods, from which one might discover an adaptive reason for geographic patterns in bill size; I concur and have resampled several populations for gizzard contents (the original 600+ samples were discarded by an entomological colleague). On the breeding grounds adults feed mostly on insects; thus, one needs to sample in winter when they feed on seeds (see maps in Grinnell and Miller [1944] for suitable study sites). Several populations with differing bill sizes are syntopic in winter, lending doubt to the effect of food choice on the evolution of bill size differences among breeding populations. Associations between bill and seed sizes might reflect adaptation as Rising expects, but experiments are needed to document that such correlations arise and are maintained because of increased fitness for individ-

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uals that choose the appropriate food size (undoubtedly an aviary experiment).

It appears that Rising's null hypothesis is that adaptation alone produces geographic differences. This view, however, seems always to recover an assumption, local adaptation, as a conclusion. Rising comments that I apparently refuse to be an adaptationist. I instead advocate an approach in which nonadaptationist explanations (e.g. environmental induction, phenotypic "drift") are tested prior to inferences from correlations about the adaptive maintenance of traits.

Morphological diversification.—Ontogenetic shifts in developmental programs are alternative mechanisms to the microevolutionary model in which phenotypic differences in size and shape arise from a gradual accumulation of mutations, each of small phenotypic effect. Variation in a few genes that control development need not be correlated with genes that encode proteins surveyed by starch-gel electrophoresis. Contrary to Rising, I discussed explicitly (1986: 94) that geographically varying phenotypes could result from (minor) genetic alterations in ontogeny. If such "minor" genetic changes effect major phenotypic change, then typical geographic variation may indeed be "noise" as Goldschmidt contended. I advocate intra-specific studies of the geography of developmental patterns, which may clarify the developmental bases of morphological differences. That is, comparisons of ontogenies of individuals from morphologically differentiated populations might establish how developmental programs differ in rate of growth and timing of onset and cessation of growth. Such comparisons should be complemented with cross-fostering studies (e.g. James 1983) to dissect genetically any bases for ontogenetic differences.

Geographic sampling of specimens.—Rising was concerned that most samples of Fox Sparrows were taken in California. Actual distances and topographic and phenotypic diversity determine the adequacy of one's sampling effort, not political boundaries. My samples included birds with bill-size differences that rival those between many avian genera. The objective was to assess genetic and morphometric variation and covariation among these samples, not to assess the range of variation in the Schistacea group as Rising implies. They were fully sufficient to document the absence of genetic differentiation (genetic variation exists within samples) in a context of significant morphometric variation (bill sizes vary over 30% between extremes). This was an unexpected result because typically we assume that morphological differentiation indicates a subdivided population structure. Other large-scale quantitative analyses of genetic population structure in birds should be conducted to assess the generality of my results, and to understand avian intraspecific genetic homogeneity.

Multiple data sets and quantitative genetics.—Rising underemphasizes the value of using a genetic data set to complement a morphometric one. Evolution

and adaptation result from processes that shape genetic variation, but we know little of the degree to which morphometric patterns in size and shape index genetic patterns (not necessarily coloration, which seems more likely to be genetically controlled). Until more studies like those of James (1983) are conducted, patterns of morphological variation should be complemented with refined molecular studies of nuclear (e.g. Quinn and White 1987) and organellar DNA (mtDNA; see Avise and Zink 1988) for which the genetics of variation is understood. Such techniques offer a more direct way to estimate levels of gene flow, and the genetic history of population differentiation. It is not easy to distinguish history and ecology as factors in geographic differentiation (Endler 1982), especially when the traits under study have an unknown degree of genetic control. Rising's caveat that morphometric characters respond to natural selection perpetuates a common misconception about the meaning of geographic differences: "One thing that it [characters having a nonzero heritability within populations] does *not* mean is that average differences in heritable characters *between* populations of the same species necessarily indicate genetic differences" (Boag and van Noordwijk 1987; italics theirs).

Both Rising and I neglected to point out that the number of independent characters represented in morphometric studies is unknown because we have insufficient information on genetic correlations between characters. For example, we treat bill depth and width as statistically discrete characters when they likely are not genetically independent (e.g. see Schluter 1984). Techniques such as principal components analysis (PCA) or MANOVA account for phenotypic, but not necessarily genetic, correlations. In contrast, proteins studied by electrophoresis are almost certainly genetically independent (a testable assumption) and thus allow useful comparisons with morphology.

If morphometric patterns alone are used, a vicariance approach in which variation in several species with common distributions is analyzed simultaneously would strengthen conclusions (e.g. Cracraft 1983). This approach allows determination of whether an extrinsic factor, such as the origin of a mountain range, has influenced intraspecific variation and yielded congruent patterns among species. In morphometric studies of single species, it is difficult or impossible to separate history from ecology.

Another bothersome aspect of morphometric analysis is that methods for estimating the magnitudes of within- and among-population variance are vague. Quantitative genetic studies that reveal the magnitude of genetic control of morphometric variation might lead to partitioning of genetic variance in morphology in a manner analogous to the F_{st} value of population genetics.

Numerical analysis.—Rising criticized the small samples of females, the analysis of which played an in-

significant role because the main sampling goal was to obtain samples for allozymic analysis, in which sexes are combined. Large sample sizes are required for robust estimates of variances and covariances; whether 10 is a magic number for reliable means is debatable. My analysis illustrated general patterns of morphometric variation. The pie diagrams might obscure some local differentiation, but because there was no allozymic differentiation, there was no reason for a finer-scale study of male morphometrics. In any event, analyses of males and females were similar and nothing was made of any differences. Rising's comment that sexual dimorphism was not mentioned indicates that he overlooked my statement (1986: 13) "males and females . . . [were analyzed separately] . . . because of known sexual dimorphism."

Since the submission of my manuscript (1984), a number of papers have appeared that involve "re-sampling" one's data to estimate the nature of unknown distributions, permitting tests of robustness of results. Examples mentioned by Rising are jackknifing (e.g. Gibson et al. 1984, Lanyon 1985) and bootstrapping (Felsenstein 1985). Rising (1988b) does not always use these techniques himself. It is possible that bootstrapping has superior statistical properties (Felsenstein 1985) and should be applied to many systematic problems, such as estimating branching diagrams (trees) and PCAs. Such analyses will no doubt reveal that "loadings" on the second and third principal components are unstable and qualify as "shape contrasts" only if sample sizes are very large (A. J. Baker pers. comm.). This will potentially affect hundreds of published papers. My PCAs of 10 individual population samples suggested that the nature of variation on PC I within populations was not the same as among populations. This has profound implications for among-population PCAs. Samples must be similarly sized (unless means are used) and very large, perhaps larger than possible for most birds, even for recovering stable estimates of eigenvalues on PC I.

Speciation.—Speciation studies have largely been narrative affairs, in part because of the difficulty of posing tests of different hypotheses. It is not clear to me that studies (especially morphometric) of geographic variation have or can be used effectively as studies of speciation. Obviously a first order problem is to agree on what species are (recently reviewed in McKittrick and Zink 1988), and then design ways of studying their origin. Is speciation simply an extension of geographic differentiation as commonly believed? Sexual selection for species-specific traits, not long-term adaptation, might be the "stuff" of speciation, but critical tests are lacking. I believe that analyses of geographic variation will play a role but I am unsure as to what that role is.

I encourage those interested in geographic variation to read Rising's thought-provoking review. I maintain that many of his comments reflect an in-

dictment based on an adaptationist world view and not a verdict reached by any conclusive analysis. Conclusions about adaptive values of geographic differences have to be made with caution, and I suggest that they will not be testable with traditional, correlational studies but with experimentation (e.g. cross-fostering). I also believe that Rising places too much emphasis on morphometric results. In fact I suggest that morphometric studies be given a back seat (*not* ignored) until the genetics of morphometric variation is established by methods of quantitative genetics.

Several challenges face students of avian geographic variation: cross-fostering studies to assess the genetic control and adaptive value of particular phenotypes, comparative studies of development of geographically different forms, assessing covariation patterns among similarly distributed species, and estimates of population structure, or the geography of genetic variation, using direct studies of DNA. A pressing problem is to discover why genetic differentiation among bird populations and species appears conservative, at least in continental species. Lastly, evolutionary biologists must reassess how geographic differentiation relates to speciation. By following some new directions, research on avian geographic variation can usher in a new era in which ornithologists contribute significantly to our knowledge of the processes that shape population-level differentiation, and ultimately speciation.

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Received 26 June 1988, accepted 1 August 1988.

Response to R. M. Zink

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Zink's study (1986) of geographic variation in the schistacea Fox Sparrows (*Passerella iliaca*) of California and Nevada provides us with an excellent opportunity to compare patterns of genetic and morphological variation in a bird species. In my review (Rising 1988a) I found much to praise, but expressed concerns about the morphological analyses and Zink's apparent diffidence about the results. My review stimulated him (1989) to discuss some of these matters in greater depth, and I take this opportunity to respond to his discussion by expanding on my concerns.

Enzymes that are identified by electrophoretic analyses are involved in cell metabolism. These have specific functions, and mutational changes may affect their biochemical effectiveness. Indeed, it seems probable a priori that most "new enzymes," if at all changed, would have reduced activity and be eliminated by natural selection. In a few cases, two or perhaps several different allozymes have optimal catalytic efficiencies in different environments, and could be retained in a polymorphic state in populations by natural selection (Koehn et al. 1983); but, for the most part, it is likely that allozymes segregating in populations are selectively equal (i.e. "neutral"). In fact the observed distributions of allele frequencies in natural populations of birds generally are not significantly different from those that would be expected if

the allozymes were neutral, their presence and frequency in populations being determined by mutation and stochastic events. Though to my knowledge no one has direct evidence relating the relative fitness of individual birds to different biochemical phenotypes, there is good indirect evidence to support a variant of the neutral hypothesis of genetic variation, the "Infinite allele-Constant mutation rate" model (the IC model; Barrowclough et al. 1985). Barrowclough et al. (1985) argue that the neutral model should be accepted as a "null hypothesis" to explain allozymic variation in birds. While I fully concur with the sentiment of such a suggestion, I have reservations about using the term "null hypothesis" in this way. A null hypothesis is a statistical hypothesis. The IC model predicts a certain distribution of alleles by frequency. The statistical null hypothesis, then, is that the observed distribution of alleles is not different from this predicted frequency. This may seem a semantic quibble, but I am concerned that people will confuse a statistical null hypothesis with that which is biologically reasonable or parsimonious. Neutral hypotheses are not always biologically reasonable, although the mutation-drift hypothesis of allozymic variation is an exception.

To give another example, on the basis of analogy with other sparrows and the theory of sexual selection, one would predict that male Fox Sparrows are larger than females, not the same size. To test this biological hypothesis, one would test a statistical null hypothesis, namely that there is no difference in size

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