Introduction: What is "The Organismal Side" of Molecular Ecology and Evolution?

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Students come to the fields of molecular ecology and molecular evolution with widely varied backgrounds and research orientations. Some are natural historians or field biologists with a longstanding affection for a particular taxonomic group such as primates, birds, or butterflies, and their goal may be to use molecular markers to better understand the ecologies, behaviors, or phylogenetic histories of their favored taxon. Others are molecular or developmental biologists perhaps well versed in the biochemistry of proteins and nucleic acids but desirous of delving more deeply into specific genes or ontogenetic pathways that underlie particular adaptations such as a fruitfly's capacity to utilize ethanol, or a primate's color vision. Other practitioners enter the field with academic backgrounds in mathematics, statistics, or population genetics, and their proclivity might be to explore the population dynamics of genes across ecological landscapes or evolutionary timescales. At their best, these and additional viewpoints merge seamlessly in the discipline that has become known as "molecular ecology and evolution."

My own academic background is itself a complex mix (Avise, 2001). I have always considered myself an outdoor biologist and naturalist at heart, with a special affinity for vertebrates and other animals. I have maintained life-long love affairs with birds and fishes, for example, but while earning advanced degrees in zoology and genetics in the early 1970s, I became intrigued also by the powerful new laboratory approaches of molecular biology. Genetic data provided by molecular methods, especially when interpreted using suitable population-genetic or phylogenetic theory, can offer fresh perspectives on many topics in ecology, natural history, and evolution, as this book will attest.

Most of the research projects described herein had their geneses in our simple desire to explore the natural biological world. But I hope they also will reflect an intellectual appreciation for "hard academic science" as a scholarly endeavor. Throughout my career, I and my students have been keenly attuned to a sobering reminder by John Platt (1964) in his famous essay on strong inference: "We (the scientific community) speak piously of taking measurements and making small studies that will add another brick to the temple of science. Most such bricks just lie around the brickyard." As Charles Darwin once stated when rejecting inductivist claims that observations should not be hypothesis-driven (Darwin, 1903): "A man might as well go into a gravel-pit and count the pebbles and describe the colors .... How odd it is that anyone should not see that all observation must be for or against some view if it is to be of any service." Thus, although most of the genetic projects described in this book at face value merely address the ecological or evolutionary details of various remarkable creatures, the wider intent of each study typically was to address some broader conceptual issue. Often, this has involved our use of the "comparative method" wherein we compare and contrast the biological parameters of interest among diverse creatures in critical attempts to address alternative genetic hypotheses about an ecological or evolutionary phenomenon. In other cases, we may have focused on the molecular minutiae of particular genetic systems such as mitochondrial DNA, or the behavioral finepoints of particular groups of organisms such as marine turtles, or the genetic details of particular ecological settings such as hybrid zones, but again a more encompassing goal was to incorporate the findings into some grander conceptual framework in ethology, ecology, or evolution.

The field of molecular ecology and evolution can be said to have two foci: organisms and molecules. The organismal side tends to focus on the descriptive use of molecular markers to address ecological or evolutionary features of the creatures themselves, whereas the molecular side tends to focus on the functional significance of genes and genomes during the evolutionary process. Although these two foci broadly overlap (because organisms are blueprinted by their genes), they nonetheless merit distinction for reasons that are both historical and heuristic.

A distinction between the descriptive and functional research paradigms in molecular ecology and evolution traces back to some of the field's pioneering publications. Thus, when Lewontin and Hubby (1966), among others, introduced protein electrophoresis to population biology in the mid-1960s, it was in the conceptual context of longstanding controversies about the magnitude and possible adaptive significance of genetic variation. Thus, much of the initial debate centered on the question: Is the newly uncovered molecular variation mostly selectively neutral or is it somehow of adaptive significance? Ever since then, a huge and vibrant branch of molecular ecology and evolution has been devoted expressly to what might be called the "Lewontonian paradigm" which inherently views the primary task for the field to be a comprehensive elucidation of the functional (i.e., mechanistic) "genetic basis of evolutionary change" (Lewontin, 1974). Across the ensuing decades, the neutrality-selection controversy has resurfaced time and again as biologists contemplated each new type of molecular data provided by the latest laboratory method. Relevant research typically proceeded on two fronts: testing various mathematical or statistical predictions of neutrality theory against estimated magnitudes or patterns of molecular variation in various species; and addressing the functional properties of particular genotypes through more direct observational or experimental analysis. The guiding question motivating this research program has always been: "What is the functional significance of molecular variation?" Today, this research paradigm continues to find expression in such burgeoning disciplines as "functional genomics," "proteonomics" (the study of proteins), "metabolomics" (the study of metabolism), and "evo-devo" (the study of developmental processes in evolution).

An equally important inventorial role for molecular variation also emerged in the late-1960s. Under this alternative research paradigm (Avise, 2004), appropriate genetic markers (whether selectively neutral or not) can be viewed as natural tracers of organismal genealogy in many ecological, behavioral, and evolutionary contexts. These natural molecular tags, which are universal to life, can help to reveal, for example, the genetic parentage of particular individuals in the wild, or the spatial genetic structure of conspecific populations, or the longer-term phylogeny of species and higher taxa. Such applications in molecular ecology and evolution epitomize the field's natural history orientation, which is guided by the question: "What can molecular markers unveil about organismal kinship, behavior, natural history, demography, and phylogeny?" In the current book, this research paradigm defines what is meant by "the organismal side" of molecular ecology and evolution. This field too has expanded dramatically, and, like its counterpart — the "molecular side" of molecular ecology and evolution — it has matured in the last few decades into an ecumenical discipline with vast conceptual as well as empirical richness.

This volume is a collection of 69 selected reprints authored by students and colleagues of the Avise laboratory, chosen to illustrate a trademark brand of research that harnesses molecular markers to the service of natural history, ecology, and evolution. The papers were chosen from among a total of more than 310 publications by Avise and his collaborators (see the Appendix) according to several editorial criteria: collectively, the articles should span four decades (from the early 1970s through the late 2000s), encompass a wide variety of molecular genetic techniques as well as taxa, and address a wide diversity of conceptual topics in genetics, ecology, and evolution; each article should be a primary research report (as opposed to a review or synthesis) and typically should come from a short-format journal such as *PNAS*, *PRSL*, *Science*, or *Nature*; and, in general, the collection should include many of the best, or most influential, or sometimes controversial, or sometimes intriguing, or otherwise entertaining "classics" to have emerged from the Avise lab over the years.

The book is arranged into eight partially overlapping sections, each dealing with a broad but recognizable conceptual arena in molecular ecology and evolution. To give readers some sense of the historical progression of the field (from the early allozyme period in the 1970s to the modern era of rapid DNA sequencing and extensive genomic analyses), publications within each section are arranged chronologically from earliest to most recent. I ask only that readers judge the merit (or lack thereof) of each paper by the technical or conceptual standards of the era in which it was published. For example, paper no. 3 (describing geographic variation in mitochondrial DNA in pocket gophers) was published nearly a decade before the word "phylogeography" was coined, and indeed it came at a time (long before rapid DNA sequencing) when restriction enzymes still had to be purified "by hand" in each laboratory because they were not yet commercially available at a reasonable cost. Similarly, paper no. 27 (on patterns of allelic expression in hybrid macaque monkeys) appeared at a time when the whole concept that gene regulation might be an important part of the evolutionary process was still quite novel. And, likewise, paper no. 36 (on genetic variation in an endangered species of pocket gopher) came at a time when very few ecologists or population biologists had given *any* serious consideration to the then-radical notion that molecular genetic information might inform conservation efforts.

Reprints in Part 1 address the magnitude and spatial arrangement of molecular variation within and among populations of diverse animal species that for various reasons have been of special interest to ecologists and evolutionary biologists. Included in this section are two seminal protein-electrophoretic surveys (among the first of their ilk on wild vertebrate species), involving a trogobitic (cave-dwelling) fish, and island and mainland populations of mice native to western Mexico and the Gulf of California. Also included in this section is the classic pocket-gopher study that introduced mitochondrial (mt) DNA analyses to population biology and thereby launched what later became known as the field of phylogeography (Avise, 2000). Additional papers in this section deal with phylogeographic patterns of mitochondrial (and sometimes nuclear) variation in catadromous eels in the North Atlantic, Red-winged Blackbirds (perhaps the most abundant native bird in North America), sea turtles (which proved to display population genetic signatures of natal homing), globally distributed seabirds (which with respect to ecology and population structure were suspected to be the avian analogues of marine turtles), and two coastal invertebrates native to the eastern United States: the Horseshoe Crab (which proved to show normal patterns of molecular variation despite its morphological status as a "living fossil"); and the American Oyster (which offered a forceful early challenge to the conventional wisdom that molecular markers are typically selectively neutral).

In Part 2, the focus shifts to genetic analyses of species that display various forms of clonality, unisexuality, or hermaphroditism. Among the clonal reproducers researched by the Avise lab are the following: sponges and corals whose population genetic architectures (including the spatial distributions of particular genets) can be revealed in their histocompatibility responses to experimental tissue grafts; various fish species that consist solely of females who reproduce by clonal (i.e., parthenogenetic) or related quasi-clonal mechanisms; and the Nine-banded Armadillo that is the only vertebrate species known to display an intra-generational form of clonality termed constitutive polyembryony (consistent "twinning"). Also included in this section are three papers on the Mangrove Killifish (the world's only hermaphroditic vertebrate species that routinely self-fertilizes), plus one paper dealing with the peculiar phenomenon of pseudohermaphroditism in a molluscan invertebrate (the Knobbed Whelk). In all of these studies, molecular markers typically helped not only to document the respective reproductive behaviors, but also to illuminate the broader ecological and evolutionary ramifications of these atypical but fascinating procreative modes.

In the papers in Part 3, highly polymorphic molecular markers (typically microsatellites) were employed to reveal biological parentage (genetic maternity and/or paternity), kinship, and mating behaviors in diverse species that reproduce by somewhat more conventional sexual means. In effect, genetic parentage represents genealogy (i.e., phylogeny) across a single generation, and such microevolutionary information accumulated for multiple breeding individuals registers the genetic mating system of a population. The reprinted paper that opens this section provided an early genetic test of one provocative hypothesis: that large schools of highly social marine fish routinely consist of close kin (perhaps even full-siblings from a single spawn). However, a critical empirical appraisal of this idea showed that juvenile red anthias fish within a school are in fact unrelated individuals (i.e., random genetic draws) from the local gene pool. Several of the reprints that follow then describe genetic parentage analyses of natural broods within various fish species in which males alone tend the nests or otherwise care for hundreds or even thousands of embryos. These genetic studies have documented a wealth of otherwise cryptic mating behaviors and reproductive patterns such as: cuckoldry via sneaked fertilization events, egg thievery, and other routes to allopaternal care of young in several fish species; instances of filial cannibalism in which a parent sometimes eats its own offspring; and, the phenomenon of sex-role reversal and its resulting altered patterns of sexual selection and sexual dimorphism in male-pregnant pipefishes. Other reprints in this section show how similar kinds of genetic parentage analyses in other brood-tending species have revealed polygamous mating systems (polygyny, polyandry, or polygynandry) in large-clutch taxa ranging from fishes to sea spiders to crayfishes. Finally, as described in another of the reprints in Part 3, a genetic parentage analysis of this same general sort was even used to uncover a remarkable physiological capability: the successful multi-year storage and utilization of sperm by wild turtle females.

The papers reprinted in Part 4 illustrate the power of molecular markers for dissecting behavioral, genetic, ecological, and evolutionary processes in cases of hybridization and possible introgression between genetically distinct populations and species. The diversity of biological topics as well as taxa addressed in these reprints is wide, ranging from patterns of gene expression in hybrid macaque monkeys to cytonuclear genetic signatures of various forms of introgressive hybridization in creatures ranging from bluegills to basses to mosquitofishes to treefrogs to oysters. Also included in this collection are papers dealing with the following: the evolutionary status of a remarkable population of hybrid catadromous eels in Iceland; the evolutionary origin via interspecific hybridization of a unisexual (all-female) fish species; and the molecular documentation of hybridization among some rather ancient lineages of marine reptiles.

In Part 5, our attention shifts to the use of molecular markers in a specific conservation context: identifying and characterizing endangered species in nature. Traditionally, conservation geneticists have been preoccupied with assessing genetic variability (heterozygosity) in species that are threatened and endangered with extinction (Frankham *et al.*, 1992), the supposition being that any observed paucity of molecular variation might severely limit a population's genetic capacity to respond to ecological or evolutionary challenges. But there are many additional roles for molecular appraisals in conservation genetics (Schonewald-Cox *et al.*, 1983; Avise and Hamrick, 1996), not the least being to assess the degree to which each "endangered species" is genetically distinct from related taxa. One of the papers included in this section was perhaps the first comprehensive molecular genetic analysis of the evolutionary and taxonomic status of an endangered species (the Colonial Pocket Gopher in Georgia). Likewise included in this set are similar kinds of genetic analyses as were applied to the now-extinct Dusky Seaside Sparrow (a former icon of the conservation movement in North America), the Kemp's Ridley and Loggerhead sea turtles (two other high-profile endangered species), a less charismatic but nevertheless rare form of freshwater musk turtle with a narrow distribution, and various other taxa of special conservation concern. The collective message from these and similar studies is that conventional taxonomic designations are not always reliable guides to the evolutionary genetic status of endangered taxa. In some cases, morphologically cryptic species worthy of conservation attention are uncovered in the genetic appraisals; but in other cases molecular analyses have confirmed the genetic distinctiveness of an endangered taxon and thereby bolstered the conservation rationale for protecting particular populations whose evolutionary status might otherwise have been in doubt.

Papers in Part 6 will remind readers that molecular markers can also be highly germane for genetic analyses of meso- and macro-evolutionary phenomena. This section begins with an early empirical genetic appraisal of the then-fresh theory of punctuated equilibrium (Eldredge and Gould, 1972), before proceeding to a host of other evolutionary topics: comparisons of molecular divergence patterns in major taxa such as birds versus other vertebrate classes; a critical genetic appraisal of an intriguing hypothesis for how green turtles might have colonized Ascension Island in very ancient times; data-motivated discussions on the concept of "speciation duration," and how the speciation process in various vertebrate groups might relate to Pleistocene phylogeographic events and to the apparent origination dates for extant sister taxa; and, finally, examples of how the procedure of "phylogenetic character mapping" (Avise, 2006) can inform our understanding of the evolutionary histories of polymorphic phenotypic traits, such as the alternative parental modes by which fishes care for their offspring, and the alternative reproductive tactics employed by males.

Part 7 consists of selected reprints in which molecular-genetic perspectives motivated the development of novel population-genetic or evolutionary theory. The broad intent is to illustrate the close interplay between data and theory that has always been one of the hallmarks of the field of molecular ecology and evolution. Some areas of theory that are highlighted in this section include: the introduction of "cytonuclear disequilibrium" statistics to describe associations between nuclear and cytoplasmic genotypes; the estimation of biological parameters such as dispersal distances and effective population sizes from the geographical arrangements of mtDNA lineages; how molecular parentage analyses can provide a genetic analog of traditional "mark-recapture" methods for estimating current-day population sizes in nature; how null alleles can impact genetic analyses of parentage; how sympatric speciation might occur via disruptive selection; how phylogeographic perspectives can inform our thoughts about species realities and speciational processes; how sex chromosomes might relate to sexually selected male ornaments in fishes; how phylogenetic appraisals might permit the first-ever attempts to universally standardize traditional Linnean taxonomic ranks; and how the use of a novel term (hemiplasy) might promote conceptual understandings and improve discourse in comparative systematics.

Finally, the papers reprinted in Part 8 illustrate how empirical discoveries from the organismal side of molecular ecology and evolution can often feed back also into improved understandings of genomic operations and molecular-level phenomena. This point is driven home beginning with a critical experimental test of strict maternal inheritance for mtDNA; additional examples from this section include: a discovery of widespread mtDNA heteroplasmy in lower vertebrates; the phylogenetic origins and molecular basis, respectively, of length variation and null alleles at microsatellite loci; the concept and documentation of clustered mutations that enter a population in cohorts (rather than as singletons as was often assumed for *de novo* mutations in traditional population genetic theory); and the discovery of the molecular phenomena of gene conversion and concerted evolution in particular regions of animal mtDNA.

Overall, I hope that the papers reprinted in this book will make interesting reading for ecologists, evolutionists, molecular biologists, and natural historians, ranging from beginning graduate students to seasoned professionals. Although this collection of papers clearly cannot substitute for conventional textbooks in the field, perhaps it can complement the latter and also offer some unique advantages. In particular, I hope that people new to the field might welcome this volume not only as a general introduction to the fascinating scope of organismal perspectives in molecular ecology and evolution, but also as offering — by way of personal examples — some professional guidance on how to conduct genetic research, construct research papers in the field, and thereby build a successful academic career — one research step at a time.

In the final analysis, I hope that the papers reprinted in this volume will be viewed not merely as loose bricks that lie scattered about a scientific construction site, but rather as integral components of a durable foundation upon which has been built "the organismal side" of molecular ecology and evolution.

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