1995

Critical Comments: Parasitology Year 2000

Albert O. Bush
Brandon University

Janine N. Caira
University of Connecticut - Storrs

Dennis J. Minchella
Purdue University

Steven A. Nadler
University of California - Davis, sanadler@ucdavis.edu

John R. Seed
University of North Carolina at Chapel Hill

J. Parasitol., 81(5), 1995, p. 835–842

CRITICAL COMMENTS

PARASITOLOGY YEAR 2000

Albert O. Bush, Janine N. Caira*, Dennis J. Minchella†, Steven A. Nadler‡, and John R. Seed§‖
Department of Zoology, Brandon University, Brandon, Manitoba, Canada R7A 6A9

ABSTRACT: We predict that in order for parasitology to thrive by the year 2000 the various subdisciplines of evolution, ecology, biosystematics, and genetics must develop holistic approaches and use parasite models to answer basic biological questions. The students of tomorrow must work as part of a multidisciplinary team; and their questions and answers must be conceptually integrated into the broader biological framework of evolution and ecology.

The old parasitology is dead. The individual investigator simply describing a new species of parasite or attempting to understand ecological relationships at the infrapopulation to community level will no longer survive at a major university or be at the leading edge of their field. The new parasitology will require an integrated approach in which the systematist, field ecologist, mathematical modeler, and molecular biochemist combine in their examination of population dynamics. To this we must add the statistician, the environmental biologist, the physical chemist, and so on. Unfortunately, no one individual can master all of these scientific specialties. Projects for the Year 2000 will have to be cooperative ones between individuals having different training, but who can work together in a team. In addition, because of the inability for any 1 department (and in many cases the University or Company) to have the resources to support the critical mass of individuals needed for such projects, proposals funded in the Year 2000 will, by necessity, involve individuals at different locations and require an exchange of information, ideas, and data via electronic communication (the Information Superhighway). Therefore, currently funded separate projects in biodiversity, biogeography, systematics, evolution, ecology, etc., will in the near future become one (the Mega Project), and the individuals involved will all have 4 basic characteristics: (1) expertise in a specific discipline; (2) a good basic understanding of general biological and chemical principles (scientific breadth); (3) the ability to work in a team; and (4) expertise in information transfer.

We have included a discussion of research in 4 major subdisciplines of parasitology that we believe will continue to be important beyond the year 2000. Each example notes the great potential of parasitological studies, the need for a multidisciplinary approach, and supports our conclusion that integrated research (and teaching) teams will enhance our discipline.

PARASITE EVOLUTION

The future of parasite evolutionary biology is a potentially bright one! It seems clear that cladistics will continue as the method of choice for the construction of hypotheses of evolutionary relationships. Most convincing will be cladistic studies executed with care from observation of a wide variety of characters from specimens representing a large proportion of the taxa involved (Deets, 1994). Such studies will continue to draw upon morphological characters obtained with light and electron microscopy (Siddall et al., 1992) In addition, as the molecular database for invertebrate taxa in general continues to expand, the utility of nucleotide sequence data for the construction of evolutionary hypotheses is becoming incontestably apparent (Hillis and Dixon, 1991). However, to date, such data have been utilized for the phylogenetic analysis of very few parasite taxa (Johnson et al., 1988; Baverstock et al., 1989; Nadler, 1992; Rohde et al., 1993). In addition, relatively few genes have been utilized as sources of sequence data (see Vossbrinck et al., 1987; Baverstock et al., 1991; Liu and Beckenbach, 1992; Nadler, 1992; Barker et al., 1993; Blair, 1993; Rohde et al., 1993). There is much to be gained from the exploration of other regions of both the nuclear and mitochondrial genomes (Simon et al., 1994) for constructing phylogenetic relationships. Research (both morphological and molecular) must also be extended to a much broader range of parasite groups.

Received 10 May 1995; accepted 10 May 1995.
* Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut 06269.
† Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47906.
‡ Department of Biological Sciences, Northern Illinois University, DeKalb, Illinois 60115-2861.
§ Department of Epidemiology, University of North Carolina, Chapel Hill, North Carolina 27599-7400.
‖ Order of authorship is alphabetical and does not imply seniority.

Whereas studies involving the generation of phylogenetic trees will themselves be valuable contributions to the parasite literature, the trees resulting from these studies will also form a framework that can be used to investigate a variety of general evolutionary principles, some of which may be most appropriate to investigate in parasite systems, as well as a plethora of intriguing evolutionary questions unique to parasite systems. Several examples follow.

Mapping the life cycles of parasites onto the phylogenetic trees of the corresponding taxa puts the life cycles into an evolutionary perspective that allows analysis of issues such as the order of origin of invertebrate (intermediate?) and vertebrate (definitive?) hosts in species with heteroxenous life cycles. For example, using this method Carney and Brooks (1991) determined that the lack of a vertebrate host from the life cycles of several species of *Alloglossidium* was not the condition that preceded the addition of the vertebrate host but rather was the result of the loss of the vertebrate host. Barta (1989) similarly identified several independent evolutionary events leading to heteroxenous life cycles among sporozoan taxa.

Related to issues of life history evolution, and equally deserving of attention, is the evolution of modes of transmission and the evolution of parasitism (Adamson, 1986). The latter line of investigation would be particularly interesting to pursue in monophyletic taxa that contain both parasitic and nonparasitic members, such as the Crustacea.

Parasite groups with larval stages that bear little morphological resemblance to their adult forms (for example many platyhelminths and acanthocephalans) are ideal systems for study of historical constraints on morphology. This is particularly interesting in parasite groups because each life cycle stage often lives in a unique environment and thus each is exposed to entirely different environmental pressures. In these taxa, phylogenetic trees can be generated independently for each morphologically distinct life cycle stage and then compared. The few studies of this nature carried out to date (Caira, 1989) indicate congruence between trees of several life cycle stages, but many more such studies are needed before the generality of this phenomenon can be assessed. One could also compare trees generated independently from molecular data collected from each life cycle stage.

Parasite systems are excellent vehicles for the study of character evolution, including frequency and type of homoplasious and nonhomoplasious character occurrences. Parasites provide excellent opportunities for the study of convergence as it occurs in response to the similar influences imposed by similar hosts, in many instances on a diversity of parasite taxa. Heterochrony is particularly attractive to pursue in parasite systems. In an excellent example of the latter type of study, Siddall et al. (1992) used the results of their phylogenetic analysis of the Diplomonadida to document the phenomenon of heterochrony in several of the ultrastructural features of this group.

Of perhaps most interest (and importance) to biologists in general are studies of host/parasite coevolution. Methods for handling host data for such comparisons include mapping hosts onto parasite cladograms (Deets, 1987) and generating host cladograms directly from parasite data (Brooks, 1981). But, by far the most informative method is comparison of trees generated independently from host and parasite data (Hafner and Nadler, 1988; Page, 1993); such studies have the advantage of independence from the assumption of strict coevolution between parasite and host taxa. Thus, future studies of coevolution must involve collaboration between parasitologists and other investigators working on the appropriate host taxa. The initiation of such collaborative studies by parasitologists may be one of the most effective ways to focus the attention of nonparasitologists on parasitological issues.

As more coevolutionary hypotheses become available, investigation of tempo (Hafner and Nadler, 1990; Page, 1991) and mode (Adamson, 1990) of speciation in both parasite and host taxa will be greatly facilitated. In many ways host/parasite associations are model systems for studies of speciation.

From the examples given it becomes obvious that studies of parasite evolution will involve the disciplines of taxonomy, biochemistry, molecular biology, statistics, biological models, ecology, etc., the key will be the integration of these separate specialties into cooperative research and teaching endeavors.

PARASITE ECOLOGY

There is much current debate on future directions in ecological research. Studies such as Weins' (1990) "Ecology 2000: An essay on future directions in ecology," Grosberg and Levitan's (1992) "For adults only: Supply-side ecology and the history of larval biology," Odum's (1992) "Great ideas in ecology for the 1990s," and Malmer and Enckell's (1994) "Ecological research at the beginning of the next century," assess past efforts and address future goals in understanding the relationship between organisms and their environments. Common to most such essays is a clear focus on problems of scale, the need for defined autecological studies on integrated groups of organisms, the need to take a holistic approach that incorporates various life history stages of organisms in those autecological studies, and the need for a forceful presentation of those ideas to an appropriate audience. Although these 4 essays have their roots in the study of free-living organisms, all can be usefully approached through studies on parasites.

The study of parasitic organisms (we focus on the parasitic protozoans and helminths, but other kinds of parasites should show similar features) provides a number of advantages not readily available to the student of free-living organisms. As organisms with complex life history patterns, parasites are excellent integrators of environmental conditions and hence are useful as variables for monitoring environmental change or ecosystem health. They also provide excellent vehicles for studying such phenomena as dispersal and dissemination in space and time. Because most are obligate symbionts, their hosts provide unambiguous boundaries within which exposure (a supply-side factor) and subsequent development (environmental limitations or interactive factors) can be measured and compared. In addition, parasites in host individuals, in host populations, and in all of their hosts, e.g., intermediate, vector, paratenic, and definitive, which harbor the parasite at some time in their life cycles provide nested levels of organization. Matters related to spatial and temporal scales are more amenable to study in such nested systems with unambiguous boundaries. An often unappreciated, perhaps forgotten, feature of the ecology of parasites is that they live in a habitat (which we call a host) that is capable of responding to their presence (and their potential pathogenesis) in a manner that may be detrimental to the parasite, to the

parasite's habitat, or to both. Unlike the free-living analogue, "environmental resistance," the response by the host involves a measurable cost, suggesting, as noted in the previous section that parasites are excellent candidates for studies on evolutionary adaptations. The availability of sophisticated physiological procedures and immunological and molecular probes sets the stage for a truly integrated, holistic, and mechanistic approach to ecological questions applied to host–parasite systems.

In North America, parasites were not really appreciated as organisms for primary studies on ecological phenomena until some of the landmark studies in the late 1950s and early 1960s. Such studies as Hairston's (1965) work on the population biology of *Schistosoma,* Noble's (1960) recognition of communities of parasites in fishes, Read's (1959) work on the ecological significance of dietary carbohydrates, Holmes' (1961, 1962) demonstration of competition, Manter's (1963) studies on zoogeography, and Schad's (1963, 1965) works on niche diversification and interference competition stimulated much enthusiasm and suggested that parasites have considerable promise as model organisms for ecological study. Unfortunately, that promise has not been realized, despite considerable advances in the various subdisciplines of parasitology.

There are probably a number of reasons why that promise remains unfulfilled. We are not suggesting that there has been a lack of good work or a lack of innovative thought on parasites and their ecology. However, most of the work that followed the landmark studies focused on inferential models (a form of "pattern analysis"), stressing the similarities in different systems. Rarely did parasitologists consider an attractive alternative—the study of adaptive differences. Although there have been numerous studies on the population dynamics of various parasites, most treated the populations as if they were in a vacuum, neglecting other parasites, the environment of the host, or especially interactions with the host. Rarely were the consequences of those dynamics (in terms of such features as selective pressures or life history strategies) pursued. A notable exception is the recent integration of modeling and field ecology as exemplified by the work of Dobson and Hudson (1992) on trichostrongyles in grouse. In spite of some exceptions, even free-living ecologists note particularly our failures. For example, in a recent article Wilbur (1990) stated "Phenotypic plasticity is one mechanism that an individual can use to survive the vagaries of a chaotic environment. Zoologists, including parasitologists. . . , have not yet exploited fully this interpretation of life history diversity."

Despite significant advances in parasite physiology and immunology, and the fact that the immediate environment of any parasite is constituted of the structure, functioning, and response of the host, there has been little incorporation of these fields in the study of the ecology of parasites. Epidemiological studies on parasites of medical and veterinary importance are an exception, but an exception that is relatively unknown to nonparasitological ecologists. Even these studies, however, often neglect the effect of other parasites in the host population.

We know a large number of details on a great many parasite species. A simple collation of addition fragmented facts will not make parasite ecology a vibrant field, capable of commanding scarce funds for basic research. Lawton (1994) voiced a similar concern when considering the field of entomology—"I believe there are major problems posed by too many different research groups studying too many different taxa, in too many different ways, with the result that we may end up not knowing enough about particular systems to say anything useful about any one of them."

Parasite ecologists need to focus on a comprehensive set of testable hypotheses, with full recognition of the directions taken by other ecologists. We need to test, using host–parasite systems, some of the basic hypotheses being worked on in free-living systems. We also need to address hypotheses that specifically test the special attributes of host–parasites systems. Such tests should focus on parasites in the "real world," as members of guilds and communities, in hosts that are members of guilds and communities. Clearly, a major goal for parasite ecologists must be to foster integrated, comparative, and collaborative studies. We would argue that group funding, in which the physiological, immunological, genetic, and ecological bases of ecological questions are addressed by teams of workers would greatly enhance parasite ecology and also ecology in general.

BIOSYSTEMATICS

Many topics involving parasites should be of interest to a wide spectrum of evolutionary ecologists. A short list of topics and citations includes the following. What effects do parasites have on the structure of ecological communities (Minchella and Scott, 1991)? How common is cospeciation versus host switching among different assemblages of hosts and parasites (Brooks and McLennan, 1991, 1993)? Does comparative analysis of host–parasite systems reveal vicariant biogeographic patterns (Hoberg, 1992; Hoberg and Adams, 1992)? Are rates of molecular or morphological evolution similar in hosts and their parasites (Hafner and Nadler, 1990; Page, 1990; Hafner et al., 1994)? Many of these research questions require comparative analysis of phylogenetic trees ("tree-thinking"). The study of host–parasite cospeciation is a particularly promising application of tree-thinking because it promotes a better understanding of the relationships between symbionts, and, in certain cases, allows investigators to gain basic insights into evolutionary processes. For example, when host–parasite cospeciation has been inferred for an assemblage, the lengths of corresponding branches in host and parasite trees can be used to assess rates of molecular evolution between the 2 groups of organisms (Hafner and Nadler, 1990; Hafner et al., 1994). Of course, the biological inference of cospeciation is not necessarily straightforward because patterns of congruence between host and parasite phylogenies can result from phenomena other than cospeciation in the strict sense (Brooks and McLennan, 1991; Page, 1993). However, given such caveats, the general features of this relatively new approach to analyzing rates of evolution will be outlined briefly as an example of one potential topic of broader interest.

Comparative studies of evolutionary rates among free-living organisms have frequently been hindered by difficulties of establishing accurate time of divergence for species. The inference of approximately contemporaneous speciation, as represented by certain host-parasite cospeciation events (Hafner and Nadler, 1990), provides an important opportunity to assess relative rates of evolution between organisms with very different life history features. Three general requirements must be fulfilled to compare rates of molecular evolution between hosts and their parasites. First, independent phylogenetic hypotheses must be gen-

erated for each group of organisms to avoid any circularity in subsequent comparative analyses. Second, comparisons of host and parasite tree topologies must reveal evidence of cospeciation, which may be inferred when levels of congruence between independently derived trees exceeds chance expectations. Finally, comparisons of analogous branches in the 2 trees necessitates using models of sequence evolution to estimate branch lengths from the sequence data.

Molecular data (particularly nucleotide sequences) are especially worthwhile subjects for comparative analysis of cospeciation because in certain cases, homologous genes can be compared between distantly related taxa to assess relative rates of molecular evolution. By contrast, morphological characters, although clearly useful for inferring evolutionary history, are much less useful for studying rates of change between distantly related organisms because no common framework of homologous morphological features is available for comparison. For molecular comparisons, protein-encoding sequences would seem to represent the genes of choice because different positions within codons, different classes of nucleotide substitutions (transitions versus transversions) and amino acid replacements can be analyzed separately. One recent study has employed mitochondrial gene sequences that have a high probability of being orthologous between hosts and their parasites (Hafner et al., 1994).

In cases where the hypothesis of cospeciation is supported, host–parasite lineages with parallel histories can be used to test the null hypothesis of equal rates of molecular change. One simple approach to assessing rates of evolution is to compare lengths of analogous branches in the host and parasite trees using a bivariate plot (Hafner and Nadler, 1990). An important part of this comparative procedure involves estimating branch lengths for taxa given a phylogenetic tree. Inferring branch lengths requires a model of nucleotide substitution, which could include maximum parsimony (minimizing substitutions over the tree), or more complex models such as maximum likelihood for which parameters such as nucleotide composition and transition bias are applied to a parametric model of substitution. Although perhaps not intuitive, a pattern of cospeciation does not necessitate equivalent rates of molecular evolution between symbionts. Molecular evolution may occur faster or slower in the parasites when compared to their hosts, and such rate differences will be revealed in the bivariate analysis as a departure from a slope of unity.

Relatively few comparative analyses of host–parasite cospeciation employing molecular data of any kind have been published, and studies using sequence data are exceedingly rare. One recent nucleotide-based study has focused on the pocket gopher–chewing louse symbiosis and sequences encoding the mitochondrial gene cytochrome oxidase I (Hafner et al., 1994). For this assemblage, maximum likelihood distance matrices were significantly correlated for host and parasite taxa that showed evidence of cospeciation. The nature of this correlation was explored further using 2 methods of analysis. Branch lengths (inferred by likelihood) for parasites were significantly longer than for their hosts as determined by Wilcoxon sign-rank tests (Hafner et al., 1994). The observation of a higher rate of substitution in parasites than their hosts was confirmed using bivariate analyses. Model II regression analysis of analogous branches in host and parasite trees showed that the overall rate of nucleotide substitution was approximately 3 times higher in the lice than their gopher hosts, which corresponded to the observed differential in amino acid replacements (on average), in pairwise comparisons of species. When the regression analysis was restricted to 4-fold degenerate sites in the cytochrome oxidase sequences (sites at which all substitutions were silent with respect to amino acid replacement), the rate of substitution was approximately 11 times higher in lice than in their gopher hosts. In theory, these synonymous substitutions, which show clocklike behavior in this case, may behave as neutral characters and reflect the underlying mutation rate. The observed difference in rates of synonymous substitutions between these hosts and parasites could be explained by several different mechanisms, including differences in metabolic rate, factors correlated with differences in body size, or as a consequence of differential vulnerability to mutation. However, if this difference reflects an underlying dissimilarity in mutation rates, then generation-time differences between pocket gophers and their lice may be an important factor. The order of magnitude difference in rates of synonymous substitution is accompanied by a similar difference in the generation times of gophers (usually 1 yr) and lice (approximately 40 days). Although additional research is needed, these results are consistent with the hypothesis that distantly related groups of animals have an equal rate of mutation per generation as inferred from silent substitution rates.

During the last 20 yr, many fundamental advances in the sciences have been made possible by new laboratory, analytical, and computational technology. This is also true for certain advances in parasitology, and thus the application of "cutting edge" research techniques is frequently viewed as a panacea for facilitating the renaissance of biosystematic parasitology as a separate discipline while simultaneously preserving positions within academic departments. However, this scenario is highly unrealistic. External granting agencies rarely fund research proposals simply because the investigation involves the application of the newest research tools. Clearly, a different approach to research must be adopted by many parasitologists if the discipline is to succeed in training a new generation of systematists while retaining an equitable proportion of academic positions. Parasitologists must also recognize that most academic departments no longer hire individuals who study alpha-level taxonomy or life cycles; instead, work in these important areas must be integrated into more comprehensive research programs in evolutionary biology, ecology, or biodiversity. Initially, this reorientation may prove difficult because the focus of parasitology research has typically concerned the intricacies of the parasites themselves rather than the development of testable hypotheses concerning general evolutionary themes. Obviously, much important research in biosystematics in general, and systematic parasitology in particular, will remain noncompetitive for external funding (arguably the keystone for retaining academic positions). On the other hand, some of this work is a prerequisite for other investigations in evolutionary parasitology and can be justified on this basis. For example, life cycle research would likely be of more widespread interest in the context of using a phylogenetic hypothesis as a framework for studying patterns of change in life cycle attributes.

Clearly, comparative studies of cospeciation using molecular data will benefit from development of new analytical approaches and additional empirical studies. However, the gopher–louse example illustrates that in addition to information on the nature

and extent of cospeciation in particular host–parasite assemblages, parasitic systems have the potential to contribute to more fundamental areas of evolutionary biology. Using parasites as model systems to investigate "big picture" issues in evolution and ecology will likely benefit from a multi-investigator approach, and collaborative research with scientists working on nonparasitic systems is likely to increase the quality and success rate of "parasitology" grant proposals.

GENETICS

This section will highlight key areas of genetic research in parasitology. Many of these studies utilize an integrated approach combining ecological/epidemiological techniques with the tools of molecular genetics. We will begin by describing studies that evaluate the potential for reduced genetic variation within laboratory-maintained parasite populations. This will lead to a discussion of studies that have assessed the amount and degree of genetic diversity within and among natural parasite populations. Next, we will summarize a study that correlates the expression of phenotypes with particular parasite genotypes. The last topic will address studies of parasite genetics at the subcellular level.

Perhaps, the most heavily funded area of research within parasitology is that related to drug and vaccine development. These studies use predominately laboratory-reared parasite populations, and yet indications are that these laboratory stains far underestimate the amount of genetic heterogeneity found in nature. An area of research that must be addressed is whether or not analysis of epidemiological processes with hypovariable laboratory stains may actually misguide pharmacological studies. For instance, among conspecific parasites that infect various host species are unique parasite alleles favored for each host species?

A study by LoVerde et al. (1985) determined that laboratory passage of *Schistosoma mansoni* through murine hosts reduces the genetic variability present in an isolate previously maintained in primates due to host-induced selection. The study involved laboratory-infected baboon populations from which miracidia were collected to infect a pool of *Biomphalaria glabrata* snails. These infected snails were then used to found 2 murine-passaged parasite populations. One snail population was 4 times larger than the other and the smaller population was intended to mimic typical bottleneck conditions associated with laboratory maintenance of the parasite life cycle. Adult worms from each generation, including the founding individuals from the baboon, were assayed for electrophoretic polymorphisms at 6 enzyme loci. Substantial temporal changes in allele frequencies at 4 of the loci were noted, with fixation of 3 of the loci occurring in 2 generations in both parasite populations. This study clearly indicated that conclusions based on laboratory-maintained populations of parasites may not directly apply to natural populations containing higher levels of genetic variation.

One feature of eukaryotic DNA that will continue to yield enlightening results for researchers is the large amount of nontranscribed repetitive sequences interspersed throughout the genome. Because they do not code for necessary products, these genes are free to change over time. Thus, the very nature of these sequences makes them ideally suited as probes in a variety of studies. Among their uses are the detection of parasites in low-level infections, the characterization of parasite species in order to determine host ranges, the identification of strains that can be correlated with behavioral patterns or epidemiological features, and the tracking of individual genotypes throughout a population.

As an example of assessing variation with a DNA probe, Minchella, Lewis et al. (1994) recently quantified intraspecific genetic differences among individual *S. mansoni* from 14 strains collected in Puerto Rico, Brazil, and Egypt using the polymorphic DNA element pSM750. Within-strain variation was quantified based on the proportion of shared bands: these values indicate that the genetic variation within laboratory strains of *S. mansoni* is generally very low. Relatively small founding populations and selection for traits that allow laboratory maintenance may increase homogeneity within laboratory parasite populations. Genetic profiles of individual parasites from the field reveal a diverse array of parasite genotypes in naturally infected intermediate hosts (Minchella et al., 1995). Clearly, it is imperative that we discern the levels of genetic variation that exist both in the laboratory and in the field.

While molecular assays like enzyme electrophoresis, restriction fragment length polymorphism (RFLP) analysis (nuclear and mitochondrial), and polymerase chain reaction (PCR) techniques are widely available for measuring genetic variation among individual parasites, their application for assessing genetic variation within and among parasite populations through population genetics methodology is just beginning. The paradigm of parasite population genetic structure as constructed by Price (1980) envisions parasite species split into many small populations with little gene flow between populations. Parasites are characterized by low intrapopulation genetic variation and high interpopulation variation. Reduced genetic variation within populations may result from nonrandom mating and genetic drift, whereas variation in selection pressures across environments maintains a high level of genetic differentiation between populations. The results of evolutionary forces are recorded in the genetic characteristics of individuals within populations and theoretical population genetics models such as Wright's F-statistics and Nei's G-statistics exist to discern between the various evolutionary forces.

It is interesting that studies incorporating these techniques to divide genetic variation among and within parasite populations sometimes yield incongruent results. The predicted result of low intrapopulation genetic variation and higher interpopulation genetic diversity has been reported among *Plasmodium falciparum,* various ectoparasitic arthropods, and several endoparasitic trematodes. However, high levels of within-population differentiation and lower levels of interpopulation genetic variation were described for supposedly isolated populations of the lone star tick *Ostertagia ostertagi* and some lung flukes. Arbitrary definition of population structure that lacks a biological basis complicates the partitioning of parasite genetic diversity within and among populations, indicating a need for a consistent ecological definition of populations. More work in this area is clearly necessary to validate the practicality of the current paradigm in parasite population genetic structure.

The epidemiology of parasitic infections relies on genetically controlled interactions between parasites and their hosts. Genetic studies ascertaining the nature of these interactions could address questions about the correlation between a parasite's

genotype and the phenotypic expression of those genes. The ability to detect DNA polymorphisms will allow us to correlate variation in phenotypic characters with accurate and independent estimates of genetic variation.

One important question of this type involves what influence the parasite genome has on epidemiological attributes of an infection; in other words, how are an infection's disease symptoms related to genetic characteristics of the infecting parasites? Questions of this sort seem particularly amenable to laboratory studies. For example, polymorphic repetitive DNA profiles of 14 North American sylvatic isolates of *Trichinella* were used to quantify genetic differences (Minchella, Eddings, and Neel, 1994). Differences in genetic profiles reflected phenotypic differences in parasite reproductive success as measured by an isolate's reproductive capacity index (RCI) in natural hosts. Parasite fecundity of 1 group (containing the R3, R9, and C34 isolates) was significantly lower than that of other isolates. A study of the impact of different genetic strains of *Trichinella* on the behavior of natural hosts, *Peromyscus leucopus,* detected variation in the type and extent of host behavioral modifications. For instance, mice infected with either *Trichinella spiralis* (pig) or R9 displayed significant decreases in exploratory activity, whereas mice infected with another sylvatic isolate (C26) actually showed increased activity levels. This type of correlational study of genetic and phenotypic characters can be taken to a finer level of resolution using molecular genetics.

Studies of parasites with complex life cycles present the opportunity to apply some of the techniques of "classical" genetics. Surely, the subset of genes controlling the growth and maturation of the malarial merozoite is quite different from the subset controlling growth and migration of the sporozoites. In the same way, essentially distinct genetic programs may be required to produce trematode cercariae, miracidia, and adult flukes. By analyzing the differential expression of genes at each of the varied points in parasitic life cycles, we will gain insight into the genetic basis underlying the host–parasite relationship.

One finding that may uncover an important mechanism for differential gene expression was made by Gunderson et al. (1987), who found 2 distinct ribosomal RNAs expressed in the sexual and asexual stages of the human malarial parasite, *Plasmodium berghei.* This study showed that 1 type of ribosomal RNA was the dominant type by at least a 20:1 ratio in sporozoites collected from the salivary glands of the mosquito host. Merozoites in the blood of the mammalian host exhibited the alternate type of ribosomal RNA, dominating by a similar ratio. If differential rates of translation are important processes that produce the distinction between sporozoites and merozoites, then the finding of different types of ribosomes during the life cycle would be an important first step toward better understanding the expression mechanism in these parasites.

In addition to searching for variations at the subcellular level, researchers are learning to manipulate some parasitic protozoa in ways that made bacteria attractive for early studies of genetics. For instance, methods have developed through which *Leishmania* mutants can be genetically complemented in a search for the function of the mutated gene. Beverley, Turco, and colleagues have developed a system for isolating 1 set of mutants on the basis of their lack of a key surface molecule (lipophosphoglycan, LPG) that has been implicated in the virulence of these parasites (Ryan et al., 1993; Shankar et al., 1993). By transfecting the mutants with a genomic DNA library and recovering the cosmids from those that regain expression of the surface molecule, it has proven feasible to work out the biosynthesis of LPG. This method of functional complementation is an exciting example of how modern genetic techniques can be used to study parasite biology.

As a final note, we would like to point out a rapidly expanding area of research in the field of parasitology, a number of genome projects currently underway. These projects begin with the creation of a low-resolution physical map and continue with the identification and sequencing of expressed genes. In the early sequencing stages, the least that these genome projects will accomplish will be to provide new potential targets for drug treatments. As they continue, they will give parasitologists the opportunity to explore further the genetic biology of parasites. Continued growth and development of genetic research utilizing parasitic organisms will help to strengthen the integration of parasitology into other biological fields.

SUMMARY

In each section, we have tried to show that parasitology has a rich history and that parasites offer many advantages over free-living organisms to scientists studying basic biological problems. However, despite many advantages, the full potential of our models has not been fulfilled or is unknown to individuals outside of parasitology. The future survival of parasitology will demand the development of hypotheses that can test basic biological questions. It will further require the integration of old tried and true techniques with new approaches in statistics, mathematical modeling, biotechnology, etc., in order to answer these questions. Finally, we believe if parasitology is to survive, the old course outlines and textbooks using primarily taxonomic descriptions must be changed. A holistic approach that incorporates the many subdisciplines of parasitology into an integrated ecological framework is required. Students must be trained in the new parasitology as well as the old; they must have a solid foundation in biology and chemistry; and they must be prepared to integrate fully into the large cooperative projects of the future. Neither the molecular biologist nor the taxonomist can stand alone. The gene sequencer adding new sequences without knowing their physiological function will add little to our basic biological understanding. The holistic ecological approach must be King for molecules to humans exist as populations within changing ecosettings and are therefore constantly evolving. It is the rules governing the interactions and evolution of these populations that we must define, and we believe this can only be approached through a team effort. Remember the old approaches to parasitology are shrinking and are in danger of being totally replaced by the "new" parasitology. However, the new can save the old for it will always be necessary to have individuals trained in classical parasite taxonomy, ecology, biochemistry, etc., within each research group.

Editor's note: These Critical Comments are based on presentations in a symposium held in conjunction with the 69th Annual Meeting of the American Society of Parasitologists in Fort Collins, Colorado in August 1994.

ACKNOWLEDGMENTS

We thank Gerald Esch and John C. Holmes. This project was begun when Gerald Esch brought the authors together and initiated discussion on the future of parasitology. When first instigated in 1992, John C. Holmes jointly authored the section on ecology. Although updated since then, the ideas in this section reflect his considerable input. The research of A.O.B. was supported by NSERA Canada. D.J.M. also thanks his students Robert Sorensen and Jason Curtis for their help in preparing the genetics section. His research program receives financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

LITERATURE CITED

ADAMSON, M. L. 1986. Modes of transmission and evolution of life histories in zooparasitic nematodes. Canadian Journal of Zoology **64:** 1375–1384.

———. 1990. Haplodiploidy in the Oxyurida: Decoupling the evolutionary processes of speciation and adaptation. Annales de Parasitologie Humaine et Comparee **65:** 31–35.

BARKER, S. C., D. BLAIR, T. H. CRIBB, AND K. TONION. 1993. Phylogenetic position of *Heronimus mollis* (Digenea): Evidence from 18S ribosomal RNA. International Journal of Parasitology **23:** 533–536.

BARTA, J. R. 1989. Phylogenetic analysis of apicomplexans of the class Sporozoea (phylum Apicomplexa Levine, 1970); evidence for the independent evolution of heteroxenous life cycles. Journal of Parasitology **75:** 195–206.

BAVERSTOCK, P. R., R. FIELKE, A. M. JOHNSON, R. A. BRAY, AND I. BEVERIDGE. 1991. Conflicting phylogenetic hypotheses for the parasitic platyhelminthes tested by partial sequencing of 18S ribosomal RNA. International Journal for Parasitology **21:** 329–339.

———, S. ILLANA, P. E. CHRISTY, B. S. ROBINSON, AND A. M. JOHNSON. 1989. srRNA evolution and phylogenetic relationships of the genus *Naegleria* (Protista: Rhizopoda). Molecular Biology and Evolution **6:** 243–257.

BLAIR, D. 1993. The phylogenetic position of the aspidobothrea within the parasitic flatworms inferred from ribosomal RNA sequence data. International Journal for Parasitology **23:** 169–178.

BROOKS, D. R. 1981. Hennig's parasitological method: A proposed solution. Systematic Zoology **30:** 229–249.

———, AND D. A. MCLENNAN. 1991. Phylogeny, ecology, and behavior: A research program in comparative biology. University of Chicago Press, Chicago, Illinois, 434 p.

———, AND ———. 1993. Parascript: Parasites and the language of evolution. Smithsonian Institution Press, Washington, D.C., 429 p.

CAIRA, J. N. 1989. A revision of the North American papillose Allocreadiidae with independent cladistic analyses of larval and adult forms. Bulletin of the University of Nebraska State Museum **11:** 1–58.

CARNEY, J. P., AND D. R. BROOKS. 1991. Phylogenetic analysis of *Alloglossidium* Simer, 1929 (Digenea: Plagiorchiformes: Macroderoididae) with discussion of the origin of truncated life cycle patterns in the gene. Journal of Parasitology **77:** 890–900.

DEETS, G. 1987. Phylogenetic analysis and revision of *Kroyerina* Wilson, 1932 (Siphonostomatoidea: Kroyeriidae), copepods parasitic on condrichthyans, with descriptions of four new species and the erection of a new genus *Prokroyerina*. Canadian Journal of Zoology **65:** 2121–2148.

———. 1994. Copepod-chondrichthyan coevolution: A cladistic consideration. Ph.D. Dissertation. University of British Columbia, Vancouver, B.C., 448 p.

DOBSON, A. P., AND P. J. HUDSON. 1992. Regulation and stability of a free-living host–parasite system: *Trichostrongylus tenuis* in red grouse. II. Population models. Journal of Animal Ecology **61:** 487–498.

GROSBERG, R. K., AND D. R. LEVITAN. 1992. For adults only? Supply-side ecology and the history of larval biology. Trends in Ecology and Evolution **7:** 130–133.

GUNDERSON, J. H., M. L. SOGIN, G. WOLLETT, M. HOLLINGDALE, V. F. DE LA CRUZ, A. P. WATERS, AND T. F. MCCUTCHAN. 1987. Structurally distinct, stage-specific ribosomes occur in *Plasmodium*. Science **238:** 933–937.

HAFNER, M. S., AND S. A. NADLER. 1988. Phylogenetic trees support the coevolution of parasites and their hosts. Nature **332:** 258–259.

———, AND ———. 1990. Cospeciation in host–parasite assemblages: Comparative analysis of rates of evolution and timing of cospeciation events. Systematic Zoology **39:** 192–204.

———, P. D. SUDMAN, F. X. VILLABLANCA, T. A. SPRADLING, J. W. DEMASTES, AND S. A. NADLER. 1994. Disparate rates of molecular evolution in cospeciating hosts and parasites. Science **265:** 1087–1090.

HAIRSTON, N. G. 1965. On the mathematical analysis of schistosome populations. Bulletin World Health Organization **33:** 45–62.

HILLIS, D. M., AND M. T. DIXON. 1991. Ribosomal DNA: Molecular evolution and phylogenetic inference. The Quarterly Review of Biology **66:** 411–453.

HOBERG, E. P. 1992. Congruent and synchronic patterns in biogeography and speciation among seabirds, pinnipeds, and cestodes. Journal of Parasitology **78:** 601–615.

———, AND A. M. ADAMS. 1992. Phylogeny, historical biogeography, and ecology of *Anophryocephalus* spp. (Eucestoda: Tetrabothriidae) among pinnipeds of the Holarctic during the late Tertiary and Pleistocene. Canadian Journal of Zoology. **70:** 703–719.

HOLMES, J. C. 1961. Effects of concurrent infections on *Hymenolepis diminuta* (Cestoda) and *Moniliformis dubius* (Acanthocephala) I. General effects and comparison with crowding. Journal of Parasitology **47:** 209–216.

———. 1962. Effects of concurrent infections on *Hymenolepis diminuta* (Cestoda) and *Moniliformis dubius* (Acanthocephala) II. Effects on growth. Journal of Parasitology **48:** 87–96.

JOHNSON, S. M. S., S. ILLANA, P. HAKENDORF, AND P. R. BAVERSTOCK. 1988. The phylogenetic relationships of the apicomplexan protist *Sarcocystis* as determined by small subunit ribosomal RNA comparison. Journal of Parasitology **74:** 847–860.

LAWTON, J. H. 1994. Something new under the sun? Oikos **69:** 177–178.

LIU, H., AND S. T. BECKENBACH. 1992. Evolution of the mitochondrial cytochrome oxidase II gene among ten orders of insects. Molecular Phylogeny and Evolution **1:** 41–452.

LOVERDE, P. T., J. DEWALD, D. J. MINCHELLA, S. C. BOSSHARDT, AND R. T. DAMIAN. 1985. Evidence for host-induced selection in *Schistosoma mansoni*. Journal of Parasitology **71:** 297–301.

MALMER, N., AND P. H. ENCKELL. 1994. Ecological research at the beginning of the next century. Oikos **71:** 171–176.

MANTER, H. W. 1963. The zoogeographical affinities of trematodes of South American freshwater fishes. Systematic Zoology **12:** 45–70.

MINCHELLA, D. J., A. R. EDDINGS, AND S. T. NEEL. 1994. Genetic, phenotypic, and behavioral variation in North American sylvatic isolates of *Trichinella*. Journal of Parasitology **80:** 696–704.

———, F. A. LEWIS, K. M. SOLLENBERGER, AND J. A. WILLIAMS. 1994. Genetic diversity of *Schistosoma mansoni*: Quantifying strain heterogeneity using a polymorphic DNA element. Molecular and Biochemical Parasitology **68:** 307–313.

———, AND M. E. SCOTT. 1991. Parasitism: A cryptic determinant of animal community structure. Trends in Ecology and Evolution **6:** 250–254.

———, K. M. SOLLENBERGER, AND C. P. DESOUZA. 1995. Distribution of parasite genetic diversity within molluscan intermediate hosts. Parasitology (in press).

NADLER, S. A. 1992. Phylogeny of some ascaridoid nematodes, inferred from comparison of 18S and 28S rRNA sequences. Molecular Biology and Evolution **9:** 932–944.

NOBLE, E. R. 1960. Fishes and their parasite-mix as objects of ecological studies. Ecology **41:** 593–596.

ODUM, E. P. 1992. Great ideas in ecology for the 1990s. BioScience **42:** 542–545.

PAGE, R. D. M. 1990. Temporal congruence and cladistic analysis of biogeography and cospeciation. Systematic Zoology **39:** 205–226.

———. 1991. Clocks, clades and cospeciation: Comparing rates of evolution and timing of cospeciation events in host–parasite assemblages. Systematic Zoology **40:** 188–198.

———. 1993. Parasites, phylogeny and cospeciation. International Journal for Parasitology **23:** 499–506.

PRICE, P. 1980. Evolutionary biology of parasites. Princeton University Press, Princeton, New Jersey, 237 p.

READ, C. P. 1959. The role of carbohydrates in the biology of cestodes. VIII. Some conclusions and hypotheses. Experimental Parasitology **8:** 365–382.

ROHDE, K., C. HEFFORD, J. T. ELLIS, P. R. BAVERSTOCK, A. M. JOHNSON, N. A. WATSON, AND S. DITTMANN. 1993. Contributions to the phylogeny of Platyhelminthes based on partial sequencing of 18S ribosomal DNA. International Journal for Parasitology **23:** 705–724.

RYAN, K. A., L. A. GARRAWAY, A. DESCOTEAUX, S. J. TURCO, AND S. M. BEVERLEY. 1993. Isolation of virulence genes directing surface of glycosylphosphatidylinositol synthesis by functional complementation of *Leishmania.* Proceedings of the National Academy of Sciences USA **90:** 8609–8613.

SCHAD, G. A. 1963. Niche diversification in a parasitic species flock. Nature **198:** 404–406.

———. 1965. Immunity, competition, and natural regulation of helminth populations. American Naturalist **100:** 359–364.

SHANKAR, A., T. K. MITCHEN, L. R. HALL, S. J. TURCO, AND R. G. TITUS. 1993. Reversion to virulence in *Leishmania major* correlates with expression of surface lipophosphoglycan. Molecular and Biochemical Parasitology **61:** 207–216.

SIDDALL, M. E., H. HONG, AND S. S. DESSER. 1992. Phylogenetic analysis of the Diplomonadida (Wenyon, 1926) Brugerolle, 1975: Evidence for heterochrony in protozoa and against *Giardia lamblia* as a "missing link." Journal of Protozoology **39:** 361–367.

SIMON, C., F. FRATI, A. BECKENBACH, B. CRESPIM, H. LIU, AND P. FLOOK. 1994. Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America **87:** 651–701.

VOSSBRINCK, C. R., J. V. MADDOX, S. FRIEDMAN, B. A. BEBRUNNER-VOSSBRINCK, AND C. R. WOESE. 1987. Ribosomal RNA sequence suggests microsporidia are extremely ancient eukaryotes. Nature **326:** 411–414.

WIENS, J. A. 1990. Ecology 2000: An essay on future directions in ecology. Bulletin Ecology Society America **73:** 165–170.

WILBUR, H. M. 1990. Coping with chaos: Toads in ephemeral ponds. Trends in Ecology and Evolution **5:** 37.

DATE OF PUBLICATION

Volume 81, No. 5, was mailed 17 October 1995