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GENETICS OF MARINE INVASIONS

GENETIC PERSPECTIVES ON MARINE BIOLOGICAL INVASIONS

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■ **Abstract** The full extent to which both historical and contemporary patterns of marine biogeography have been reshaped by species introduced by human activities remains underappreciated. As a result, the full scale of the impacts of invasive species on marine ecosystems remains equally underappreciated. Recent advances in the application of molecular genetic data in fields such as population genetics, phylogeography, and evolutionary biology have dramatically improved our ability to make inferences regarding invasion histories. Genetic methods have helped to resolve longstanding questions regarding the cryptogenic status of marine species, facilitated recognition of cryptic marine biodiversity, and provided means to determine the sources of introduced marine populations and to begin to recover the patterns of anthropogenic reshuffling of the ocean's biota. These approaches stand to aid materially in the development of effective management strategies and sustainable science-based policies. Continued advancements in the statistical analysis of genetic data promise to overcome some existing

limitations of current approaches. Still other limitations will be best addressed by concerted collaborative and multidisciplinary efforts that recognize the important synergy between understanding the extent of biological invasions and coming to a more complete picture of both modern-day and historical marine biogeography. [**Reviewer Comment: The abstract is fine in terms of content but it is written in a rather dense style. For example, the first sentence/two sentences could read more simply as “By moving organisms around the planet, humans have had profound effects on distribution patterns. The extent of this human influence remains underappreciated in the ocean, in part because it can be difficult to unambiguously distinguish native and invasive species. Genetic methods can help in this regard because...” This is just an example, but try to rewrite with simple sentences and minimal jargon.**]

Key Words invasive species; molecular genetics; cryptic species; multiple introductions; cryptogenic species

I. INTRODUCTION

The last few decades have seen increasing concern for the state of the ocean’s biota ([Pew Oceans Commission 2003](#), [US Commission on Ocean Policy 2004](#)). A growing list of stressors has led to extensive changes in the distribution and composition of species on the world’s coastlines, in the ocean’s water column, and even on the deep sea floor. This review deals with one of those stressors, human-assisted marine biological invasions, defined as the establishment of species in geographic regions outside the reach of their natural dispersal mechanisms ([Rilov & Crooks 2009](#)).

As an agent of change, marine biological invasions differ in some ways from other threats, such as anoxic dead zones, habitat destruction, overfishing, rising water temperatures, or acidification. These are all direct causes of removal of individuals or species from a region through extraction, death, or failure to reproduce or recruit. In contrast, biological invasions can fundamentally modify communities by the addition of novel species. These additions can lead to unprecedented predatory, competitive, or disturbance networks, which can in turn---and in concert with other agents of change--- lead to the removal of individuals or species, the latter including both native and previously introduced taxa ([Carlton & Ruiz 2005a](#), [Steneck & Carlton 2001](#)). Invaded communities are often so dramatically and irreversibly altered that economic and social values are degraded;

accordingly, the prevention of invasions has become a high priority for governments worldwide ([Hewitt et al. 2009](#)).

Mandates to understand the extent of invasions, eradicate existing invasions, or prevent new invasions have led to the sobering realization that our knowledge of marine life is woefully inadequate. For many regions of the world, we cannot even produce a simple list of which species are introduced: Thousands of species cannot be labeled as native or nonnative due to a lack of systematic, biogeographic, and historical data ([Carlton 2009](#)). Further, when a new invasion is detected, identification is often problematic. Knowledge of sources (which are linked to vectors) and routes ([Carlton & Ruiz 2005b](#)) for recent invaders can be crucial to stemming the flow of new invaders, yet without accurate species identification these sources and routes are not knowable. Even when a new invader is identified, the list of plausible geographic sources may be large and we may lack criteria to choose among them. Frustratingly, invaders that are seemingly well-known taxonomically often dissolve into mystifying complexes of species or clades when examined more closely. In short, the modern crisis in systematics, that is to say, the decline in the number of classical systematists focused on particular taxa ([Boero 2001](#), [Ehrenfeld 1989](#)), has left us with a startlingly incomplete picture of marine biodiversity and its geography at the species level at a time when the stressors[**AU: OK?] upon marine life---including the number of invasions---have been steadily increasing.

The huge gaps in systematic knowledge will not be filled fully by classical, morphology-driven systematics, as valuable as contributions from that field have been and will continue to be. For this reason, invasion biologists increasingly employ genetic methods to study invaders, seeking to answer the questions posed above: Who are the invaders? where did they come from? and how did they get here? Genetic data can strongly enhance, and in some cases would appear to have many advantages over, traditional morphological information. First, in principle, biological species (almost all of which are defined solely on morphological criteria) should be distinguishable at the genetic level from others, given sufficient evolutionary time since divergence from sister taxa. Second, methodology for genetic analysis is far more uniform than for morphological analysis, and often more quickly done. This reduces the bottleneck of finding taxonomic specialists, if such exist.

Third, genetic variation within species can potentially have a stronger geographic signal than morphological variation, making assignment of probable sources possible.

This paper aims to assess both the potential and the limitations of genetic approaches for addressing these issues. Much progress has been made in the application of molecular genetic methods to marine bioinvasions since [Holland \(2000\)](#) published his review of the topic a decade ago. Indeed, several reviews have been published recently that deal with specific aspects of marine invasion genetics, typically focusing on particular taxa (e.g., seaweeds [[Booth et al. 2007](#)] and fish [[Hanfling 2007](#)]) or particular issues (e.g., the relationship between genetic diversity and invasion success [[Roman & Darling 2007](#)]). Here we seek to build on this work, principally by exploring the utility of genetic methods for understanding the diversity and biogeography of marine invasive species. Such genetic methods help to reveal the patterns and processes of marine bioinvasion, and thus contribute directly to the design of effective management strategies and the development of sound policies at regional, national, and international levels. Equally important, they also lead to a growing appreciation of the need to better comprehend the rapidly changing diversity and distribution of marine biota, and they provide crucial insights to drive an enhanced general understanding of biogeography in the world's oceans.

II. DETECTION OF INTRODUCED SPECIES

[**AU: AR House Style calls for intervening text between headings. Pls. supply a paragraph here to introduce the following sections.**]

A. The Problem of Cryptogenic Species

Many introductions are obvious. In the nineteenth century, large numbers of commercial oysters (*Crassostrea virginica*) were moved from the North American Atlantic coast to San Francisco Bay and to many other North American Pacific coast estuaries. Along with these oysters came, unintentionally, well-known, conspicuous, and large mollusks such as the Atlantic oyster drill *Urosalpinx cinerea* and the Atlantic marsh mussel *Geukensia demissa* ([Carlton 1979b](#)). Such introductions, when directly linked in space and time to well-known vectors, are the “low hanging fruit” of invasion biology. Thousands of similar species-and-

vector-specific case histories occur in terrestrial, freshwater, and other marine locations worldwide. But for thousands of other taxa in the world's oceans, ranging from bacteria to fungi, and from protozoans to polychaete worms to seaweeds, determining which species may have had their interoceanic and transoceanic distributions radically altered by human activity in the past 1000 or more years remains a profound challenge. As a result, shores[**Reviewer comment: Give examples here: such as ...**] that have sustained invasions by hundreds of species are still regarded as having a mere handful of nonnative species ([Carlton 2009](#)), due to the lack of resolution and understanding of regional systematics, historical biodiversity and biogeography, and evolutionary biology.

Criteria have been proposed ([Carlton 1979a, 2009](#); [Chapman & Carlton 1991, 1994](#)) as first-order solutions for determining whether a given species is native or introduced (see sidebar). Despite the robustness of many of these criteria, data are often lacking for many taxa. Soft-bodied animals and plants do not preserve well if at all in the archeological and paleontological record, and even those organisms with skeletons may be differentially preserved. Convincing historical evidence of prior absence is often lacking for a vast number of poorly studied groups. A great many species of invertebrates and algae are still considered to be “cosmopolitan” in distribution, despite the clear impossibility of gene flow in ecological time, and despite the mounting evidence that virtually all such species, once struck with the molecular hammer, dissolve into multiple species complexes. The systematics and taxonomy of many invertebrate groups thus remain poorly known or unknown, making the linking of a particular species to a distant clade in another ocean of little or no value. Many species also cannot be linked to a particular vector in time and space. Further, ecological associations may be challenging: demonstrably native species can co-occur with introduced species, and native species occur in (but are usually not restricted to) highly disturbed, human-created habitats as well.

As a result, a great many species---we estimate that there are thousands [**AU: Annual Reviews does not use italic or bold for emphasis, so I will replace such instances with regular face.**]in the oceans alone---are cryptogenic: They cannot be resolved as either native or introduced ([Carlton 1996](#)). It is in the domain of evolutionary biogeography that genetics can help resolve the status of potentially many **cryptogenic species**. We have a reasonably good understanding of the evolution of modern marine biotas ([Vermeij 2001](#)),

which allows us to make some predictions about the phylogenetic relationships of a species taken from a given biogeographic region: Its nearest relatives should be found in the same geographic region, or there should be a plausible and known biogeographic and historic relationship (usually between adjacent regions) among closely related species.

Cryptogenic species: a species for which the evidence is insufficient to determine whether it is native or introduced

Mussels in the genus *Mytilus* are a good example but also serve to illustrate that certain approaches and levels of resolution require that a species group consist of relatively recently diverged clades, and ideally, a good fossil record. While the genus and many of its species have a tortured history of splitting and lumping ([Soot-Ryen 1955](#)), the mussels inhabiting bays (and sometimes exposed coasts) worldwide were long considered a single species, *Mytilus edulis*. In retrospect, a temperate, cosmopolitan, and harbor habitat should have been considered suspicious. Genetic analysis, first with allozymes ([McDonald & Koehn 1988](#), [McDonald et al. 1991](#)) and later with DNA ([Geller et al. 1994](#), [Hilbish et al. 2000](#), [Rawson & Hilbish 1995](#)), showed that there were at least three species in this group, *M. edulis*, *M. galloprovincialis*, and *M. trossulus*, the last two names having long existed but submerged into synonymy with the first. *Mytilus edulis* by definition was confined to the North Atlantic; *M. galloprovincialis* was rooted in the Mediterranean but with disjunct populations in North America, South Africa, Japan, Australia, New Zealand, and elsewhere; and *M. trossulus* was restricted to the Northeastern Pacific but occurring in the North Atlantic as well.

Phylogenetically, *M. trossulus* is basal in the group. In a natural Tertiary transarctic dispersal event, *M. trossulus* invaded the North Atlantic Ocean, where it evolved into *M. edulis*. *M. galloprovincialis*, sister to *M. edulis*, and the youngest member of this lineage, then evolved in the Pleistocene in the Mediterranean Sea ([Rawson & Harper 2009](#)). *Mytilus trossulus* later reinvaded the North Atlantic through a much later Pleistocene transgression ([Rawson & Harper 2009](#)), explaining its presence there, while *M. galloprovincialis* later exited the Mediterranean and moved north along the European coast, perhaps with some measure of human assistance.

The seemingly anomalous occurrences of *M. galloprovincialis* or *M. galloprovincialis*--like mussels in the South Atlantic Ocean and the Pacific Ocean appear to be a combination

of natural interoceanic dispersal processes and human-mediated invasions, not all of which have been adequately sorted out. Human-mediated invasions of *M. galloprovincialis* in the twentieth century (if not earlier) established the species on the North American Pacific coast, Japan, South Africa, Chile, and western Australia ([Gerard et al. 2008](#)). On the other hand, it appears that ancient *M. galloprovincialis* stocks naturally invaded the South Atlantic and South Pacific between 0.5 mya and 1.3 mya, resulting in three distinct southern clades in South America and the isolated Kerguelen Islands, in New Zealand, and in Tasmania ([Gerard et al. 2008](#)), and upon one or more of which species southern hemisphere Latin names (of which there are a number of choices, [Soot-Ryen 1955](#)) should probably be bestowed.

The kelp-dwelling bryozoan *Membranipora membranacea* is a good example of a species with a cosmopolitan but suspiciously disjunct distribution, making it potentially treatable as cryptogenic and a good candidate for investigation as an invasive species in some locations. Molecular phylogenetics, however, show that *Membranipora* from different oceans fall into monophyletic clades, and there is no local intermingling of haplotypes from unrelated clades ([Schwaninger 2008](#)): *Membranipora* “*membranacea*” thus appears to be a cluster of distinct native species in different regions. The clear exception is a recently established population of *M. membranacea* in the Northwest Atlantic Ocean, genetically indistinguishable from European populations and considered to be introduced as planktotrophic cyphonautes larvae by ballast water ([Schwaninger 1999](#)).

One pitfall of the biogeographic approach is that there may be insufficient phylogenetic signal to discern the relationships among species inhabiting different biogeographic regions. A second pitfall is undersampling; a great many genetic studies undersample native populations, leading to weak or even wrong conclusions. These difficulties ensure that the determination of nonnative status by genetic means will not be without controversy. One prominent example involves the periwinkle *Littorina littorea*, a common snail in Europe that became abundant around Halifax, Nova Scotia in the 1850s and then spread as far south as New Jersey by 1880 ([Carlton 1982](#)). Because a large, edible intertidal snail would be difficult to overlook, this record suggests a recent introduction, an interpretation that assumes putative fossil shells of *L. littorea* associated with archeological camp sites are actually of a post-European contact vintage ([Chapman et al. 2008](#), [2007](#)). [Wares et al.](#)

(2002), however, reported higher than expected genetic diversity in North American populations of *L. littorea* as well as the presence of many “uniquely” North American haplotypes of COI. Those authors used a coalescent approach (MDIV, [Nielsen & Wakeley 2001](#)) to estimate that populations of *Littorina littorea* in North America had diverged from those in Europe minimally 8000—16,000 years before anthropogenic transport was likely. They thus concluded that it was native; putative fossils were accepted as authentically prehistoric[**Reviewer comment: Never tested with dating?**.].

Subsequent statistical analysis by [Chapman et al. \(2007\)](#) suggested that genetic diversity in Europe had been undersampled, and that the presence of uniquely North America haplotypes in the limited available dataset could not exclude a recent European origin of those haplotypes. These authors also point out that programs such as MDIV contain assumptions (such as equal effective population sizes among sampled populations) that may be violated in specific cases (especially those that involve invasion). Notably, [Chapman et al. \(2007\)](#) and [Cunningham \(2008\)](#) both reanalyzed [Wares et al.'s \(2002\)](#) data and came to different conclusions about minimum time of divergence of European and North American populations, in part due to differences in parameterization of the program. Only after expanding sampling in Europe and North America were [Blakeslee et al. \(2008a, b\)](#) ultimately able to settle the argument. That study adopted multiple approaches to estimate haplotype diversity (showing American populations to be tenfold lower), examined divergence between regions using software (IM, [Hey & Nielsen 2004](#)) that makes no assumption about population size (showing divergencies in the 100s, not 10,000s of years), and observed no uniquely American subclades. In addition, [Blakeslee et al. \(2008a, b\)](#) substantiate the value of parsimonious inference based on multiple lines of evidence ([Chapman et al. 2008](#)) by including corroborating genetic data from a host-specific trematode parasite found in *L. littorea*. Their results suggest that both *Littorina* and its parasite were introduced to North America <1000 years ago.

An object lesson from this case history is that adoption of the latest theoretical approaches has the benefit of conceptual advances, but also has the cost of being untested by a body of casework that can expose the sensitivity of the analysis to its underlying assumptions. Interestingly, [Brawley et al. \(2009\)](#) have subsequently concluded that historical trade patterns, combined with genetics, further confirm that both *Littorina littorea*

and the European seaweed *Fucus serratus* were introduced to Nova Scotia in the late eighteenth or early nineteenth century, perhaps from Ireland and Scotland.

III. CRYPTIC INVASIONS

[**AU: AR House Style calls for intervening text between headings. Pls. supply a paragraph here to introduce the following subsections.**]

A. The Importance of Cryptic Invasions

The term cryptic invasion has come to capture a wide variety of phenomena ([Table 1](#)). This broad use of the term can lead to some confusion, particularly when the concept of cryptic invasions overlaps with the potentially related concept of **cryptic species**. [**AU: These green terms will be hyperlinks in the online version of your article. They will be not bold/colored in the print version. The definition just below will appear in the margin of the print version.**] The latter are typically understood as multiple, distinct biological species that have been mistakenly characterized as a single species, most often because closely related but genetically distinct clades can be difficult to distinguish morphologically ([Bickford et al. 2007](#)). [**AU: Do you wish to work the term “morphospecies” into the text here? It is one of the Terms and Definitions ([see just below](#)), but it does not appear (other than in the definition of cryptic species below) again until Table 1 and we cannot hyperlink table entries. Otherwise, you may elect not to include the definition “morphospecies.”**] A cryptic invasion, on the other hand, is any invasion event that goes unrecognized because the invader is mistaken for something else, which could be anything from a native species morphologically difficult to distinguish from the invader [[Carlton \(2009\)](#) has referred to introduced species misidentified as previously known or unknown native species as pseudoindividues] to an overlooked, distinct evolutionary lineage of a single invading species.

Cryptic species: a single morphospecies comprising one or more biological species (genetically distinct and theoretically/demonstrably reproductively isolated)

Morphospecies: a species defined entirely on the basis of morphological characteristics[**AU: See query above: Delete this term?**]

<COMP: PLEASE INSERT TABLE 1 HERE>

Thus the concept of cryptic invasions can also contain the concept of **multiple introductions** (Table 1). Multiple introductions describe cryptic invasions at the intraspecific level---in other words, multiple invasion events by the same species that are only recognized through direct observation or genetic analysis. Indeed, the continuity between the two phenomena of invasion by **cryptic clades** (i.e., multiple introductions) and invasion by **cryptic species** is seamless enough that in some cases it may be impossible to distinguish between the two based on genetic criteria alone (see below).

Cryptic clades: morphologically indistinguishable monophyletic lineages embedded within a single biological species

Multiple introductions: introductions of a single biological species to a single recipient region from two or more genetically distinct sources

The recognition of **cryptic invasions**, whether at the inter- or intraspecific level, represents a critical challenge to invasion biologists. Most obviously, the mistake of collapsing multiple invasion events into one will lead to an underestimation of the frequency with which invasions have occurred, and will prevent a full accounting of the likely routes and vectors of introduction to a recipient region. Critically, failure to recognize cryptic diversity, particularly at the species level, may lead to erroneous assumptions regarding the ecological or genetic characteristics of invasive populations, with potentially calamitous consequences for risk assessment. On a more fundamental level, without understanding the full extent to which contemporary marine communities have been shaped by introduced populations, it is impossible to adequately understand the ecological and evolutionary mechanisms driving patterns of community structure (Carlton 2009). Taken together, these considerations illustrate how the failure to distinguish cryptic invasions helps sustain continued underestimation of the ecological impacts of biological invasions.

Cryptic invasion: an invasion that remains unrecognized because the invader is mistaken for native or previously introduced cryptic species or clades

Although the challenge of identifying cryptic diversity is universal, there is reason to believe that the problem may be particularly acute in marine habitats. The ability to detect

species differences may be hampered by the decoupling of ecological and morphological divergence in some marine phyla, and the predominance of chemosensory recognition systems in the sea may allow evolutionary diversification without the morphological correlates typically useful to taxonomists ([Knowlton 1993](#)). Perhaps most important, the notion of unimpeded dispersal in marine environments may predispose researchers to assume genetic uniformity of widespread marine populations, or to accept uncritically the idea of “cosmopolitan” species with extremely broad “natural” distributions ([Carlton 2009](#), [Knowlton 1993](#), [Palumbi 1994](#)).

B. Invasions by Multiple Cryptic Species

Fortunately, the growing accessibility of molecular genetic tools has greatly facilitated the assessment of cryptic biodiversity ([Miura 2007](#)). In particular, the past five years have seen a flurry of genetic studies identifying invasions of cryptic marine species ([Table 2](#)). As indicated in [Table 1](#), these can be divided into those studies that utilize genetic methods to distinguish between described but morphologically similar sibling species and those that use these methods to infer the existence of previously unrecognized species. In the former category, for instance, [Geller et al. \(1997\)](#) reported mitochondrial haplotypes of both the western European green crab *Carcinus maenas* and its sibling species the Mediterranean *C. aestuarii* from introduced populations in South Africa and Japan. These had previously been attributed solely to *C. maenas* and *C. aestuarii*, respectively. Similarly, molecular methods detected the introduction of the Asian red alga *Polysiphonia harveyi* in New Zealand, previously mistaken for the morphologically indistinguishable native *P. strictissima* ([Mcivov et al. 2001](#)). That marine taxa poorly resolved by external morphology are so common has likely resulted in a large number of invasive populations misidentified as either other nonnatives or morphologically similar native species ([Carlton 2009](#)).

<COMP: PLEASE INSERT TABLE 2 HERE>

Far more common are studies reporting novel genetic diversity consistent with the existence of multiple undescribed species. In some cases, widely distributed taxa previously designated as single species are found actually to comprise sibling species complexes. Thus, the cosmopolitan tunicate *Ciona intestinalis* is in fact two species, each having achieved different global distributions through anthropogenic dispersal ([Caputi et al. 2007](#)). The widely distributed moon jelly *Aurelia aurita* also consists of multiple cryptic species, only

one of which has proved widely invasive thus far ([Dawson et al. 2005](#)). Most often, however, genetic analysis of an invasive population reveals unexpected genetic diversity sufficient to suggest that multiple species have been introduced---even if those putative species have not been satisfactorily described from their presumed native range (category C in Tables 1 and 2). In such cases, investigation of invasive populations may in fact raise taxonomic issues unaddressed by research in the source range of the taxon.

The observation of cryptic species diversity in invasive populations may offer crucial insights into the structuring of ecological communities. The recognition that blue mussels of California belonged not to the “cosmopolitan” species *Mytilus edulis*, but rather to the California native *M. trossulus* and the Mediterranean *M. galloprovincialis* ([Geller et al. 1994](#), [McDonald & Koehn 1988](#), [Suchanek et al. 1997](#)), allowed [Geller \(1999\)](#) to reason that the native mussel had been largely (and without being noticed) replaced in southern California by the invasive Mediterranean species in the twentieth century. In addition, a number of studies have revealed relevant ecological differences between identified cryptic lineages, potentially supplying critical information for assessing the likely impacts of marine invasions. [Davidson & Haygood \(1999\)](#), for example, described cryptic species of the bryozoan *Bugula neritina* in California that harbor different strains of associated gamma-proteobacteria. These symbionts in turn produce different forms of bryostatin, a class of cytotoxic compounds that confer chemical protection to the bryozoan larvae, raising the possibility that different species of *Bugula* may exhibit differential susceptibility to predation. In turn, at least one member of the *neritina*-clade is a widely distributed, ship-dispersed harbor species. In Europe, invasive populations of the North American polychaete *Marenzelleria* derive from two cryptic lineages, one of which seems to exhibit higher salinity tolerance than its sibling ([Bastrop et al. 1998](#)). Similarly, the widely introduced hydroid *Cordylophora* comprises at least two major lineages divergent enough to warrant species status; while one of these is restricted to brackish habitats, the other is primarily a freshwater taxon ([Folino-Rorem et al. 2009](#)).

In all of these cases, ecological observations provide evidence that the cryptic diversity uncovered by genetic methods may be relevant to understanding the impact of different invasive lineages. Unfortunately, in most cases reports of cryptic species diversity associated with marine invasions are accompanied by little additional guidance regarding

the taxonomy, biogeography, and ecology of these recently identified lineages. Cryptic diversity is, naturally, most often discovered in taxa for which limited genetic information exists from native populations, and few investigations of invasive populations can mount the necessary sampling to conduct full biogeographic assessments of native distributions. Moreover, this limitation, along with the lack of recognizable diagnostic characters, a history of association between the taxa in question and anthropogenic dispersal vectors, and the possibility of anthropogenic alterations to native habitats and turnover of native populations may precipitate a perfect storm of taxonomic confusion demanding sustained collaborative and multidisciplinary efforts to effect resolution.

C. Multiple Introductions

The role of **multiple introductions** in facilitating biological invasions has garnered substantial recent attention. In part, this is due to the recognized importance of propagule pressure to the establishment success of introduced populations ([Lockwood et al. 2005](#)), and to the perception that avoidance of genetic bottlenecks through influx of additional diversity may be critical to invasion success ([Dlugosch & Parker 2008](#), [Roman & Darling 2007](#)). However, a number of studies have also indicated that multiple introductions can have substantial impacts through the conversion of among-population genetic diversity in the native range to within-population genetic diversity in the introduced range. The consequent emergence of novel allelic combinations has been demonstrated in some cases to drive phenotypic diversification and alter the adaptive potential of invasive populations ([Facon et al. 2008](#), [Kolbe et al. 2007](#)).

Genetic analyses recently have revealed a number of marine invasions deriving from multiple introductions ([Table 2](#)). In some cases, these analyses provide novel explanations for range expansions previously attributed to alternative mechanisms. The shore crab *Carcinus maenas* had been established in the Northwest Atlantic for almost two centuries prior to observations of rapid northward expansion along the Scotian shelf in Canada. Although this change was ascribed by some to increases in northern ocean temperatures conducive to larval recruitment, [Roman \(2006\)](#) elegantly demonstrated that it resulted instead from a second cryptic introduction beyond the previous northern range limit of the species. The spread of the gastropod *Cyclope neritea* in France has similarly been attributed to unnoticed multiple introductions from native sources ([Simon-Bouhet et al. 2006](#)).

For many marine species, substantial phylogeographic structure in the native range allows multiple introductions from populations with ecologically relevant physiological differences. The consequences for management and risk assessment of recognizing multiple introductions can in these cases be similar to those associated with cryptic invasive species. For instance, separate introductions of the invasive red alga *Polysiphonia harveyi* to the North Atlantic likely derive from independent native lineages, one associated with populations on the cold-temperate Japanese island of Hokkaido and the other associated with populations from the warm-temperate Honshu ([Mcivor et al. 2001](#)). Multiple ecophysiological characteristics, including tolerance to both temperature and turbidity, may differentiate invasive lineages of *Caulerpa taxifolia* in the Mediterranean, as genetic evidence implicates independent introductions of both an inshore-mainland lineage and an offshore-island lineage from the native Australia ([Meusnier et al. 2002](#)). In one interesting case, colonization patterns by divergent lineages of the amphipod *Gammarus tigrinus* introduced to Europe from the Northwest Atlantic suggest evolved differences in salinity tolerance ([Kelly et al. 2006a](#)). What is particularly intriguing about this case is that genetic analyses indicate possible admixture between invasive lineages at recently invaded sites; whether or not this has resulted in the emergence of novel genetic types with altered invasive characteristics awaits further study.

A key conclusion is that the longtime management argument that less attention needs to be paid to continued importations of stocks of a species already established in one's country is not well supported. Such importations may in fact involve new clades of what are ostensibly the "same species," but which in fact can have strikingly different physiological or ecological characteristics.

D. Methods of Inferring Cryptic Invasions

Studies describing **cryptic invasions** may vary widely in terms of the taxa investigated and the particulars of the taxonomic murkiness involved, but the methods of inference are often similar, with differences attributable largely to whether resolution is sought at the intra- or interspecific level. By far the most widely adopted method is phylogeographic reconstruction based on DNA sequence data. Inference of phylogenetic relationships between samples, either through construction of true phylogenetic trees or haplotype networks, may reveal signatures of evolutionary divergence between two or more lineages;

this, combined with observation of the geographic distribution of genetic diversity in both the introduced and native range, can prove a powerful method for determining the presence of cryptic species, the likelihood of multiple introductions, or both.

Two phylogeographic patterns can be particularly informative for inferring cryptic invasions (**Figure 1**). First, **reciprocal monophyly** is strongly suggestive of independent evolutionary lineages within a genetic dataset; if different invasive samples can be shown to derive from reciprocally monophyletic clades, this provides powerful evidence for multiple cryptic invasions. Second, marked genetic structure within the native range of a taxon can support the hypothesis of cryptic invasions even in the absence of reciprocal monophyly. For instance, the presence in an invasive population of haplotypes with nonoverlapping distributions in the native range (with the caveat that sampling must be sufficient in the native range to reliably document nonoverlapping distributions) may suggest multiple introductions from genetically distinct sources. In ideal situations, both of these conditions may hold ([Caputi et al. 2007](#)); in some cases, even moderate genetic structure in the native range may allow inference of multiple introductions despite poor phylogenetic resolution ([Roman 2006](#)); in the worst cases, weak phylogeographic signal in the native range may severely limit the ability to draw any inferences regarding cryptic invasions.

Reciprocal monophyly: in a phylogeny, when all members of a lineage share a more recent common ancestor with each other than with any other lineage

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<COMP: PLEASE INSERT FIGURE 1 HERE>

Figure 1 Phylogeographic patterns indicative of cryptic invasions. (a) A fictitious phylogeny inferred from DNA sequences generated from an invasive population, along with an appropriate outgroup. Bootstrap support is shown for major clades, as well as a scale bar indicating percent sequence divergence. Two well-supported monophyletic clades are present in this population, suggesting that multiple evolutionarily distinct lineages have invaded the region. Whether or not these lineages represent cryptic clades of the same species or cryptic species is a matter for integrated taxonomic assessment. The observed genetic divergence between clades (approximately 5%) suggests the possibility of differentiation at the species level. (b) Fictitious haplotype frequency map for a western Pacific taxon introduced to the San Francisco Bay region. The presence in San Francisco Bay of haplotypes with nonoverlapping geographic distributions in the native range (red

and blue) suggests multiple independent introductions.[**Reviewer comment: Is there some connection between A and B in this figure? Could there be? Would it be better to split them?**]

The utility of the phylogeographic approach depends in large part on whether the genetic loci queried exhibit rates of evolutionary change suitable for the level of taxonomic resolution sought. It should come as no surprise, then, that most studies describing cryptic invasive species have adopted loci now commonly employed in DNA “barcoding”; the mitochondrial *cytochrome c oxidase subunit I* (*COI*) gene, a barcoding standard, has been especially popular (18 of 35 studies in Table 2). The espousal of these loci as DNA barcodes is based explicitly on the observation that their rate of evolutionary change often results in nonoverlapping distributions of inter- and intraspecific genetic variation, allowing unambiguous assignment of individuals to species level in a wide variety of taxa (Hebert et al. 2003).

Nevertheless, the utility of any particular locus is likely to vary from taxon to taxon. For instance, the mitochondria of anthozoans and sponges evolve at a far slower rate than that of other animal phyla, rendering mitochondrial loci useless for reconstruction of phylogenetic relationships at the subspecies or even species level (Duran et al. 2004, Shearer et al. 2002). For anthozoans and sponges, ribosomal internal transcribed spacer (*ITS*) regions have been fruitfully applied (Duran et al. 2004, Ting & Geller 2000). Concepcion et al. (2008) developed a novel sequence-based marker displaying the appropriate level of variation across samples to recognize cryptic diversity among invasive species in the anthozoan genus *Carijoa*. More generally, it has become commonplace for researchers to assess multiple loci, often from different genomes (nuclear, mitochondrial, or plastid) in order to guard against the possibility of any one locus leading to mistaken phylogenetic inference. This approach enabled Meusnier et al. (2002) [**AU: Confirming that this cite is to the Meusnier “et al.” reference in Lit. Cited section? See also at p. 18.**] to detect strong support for reciprocal monophyly at the *ITS* locus of the highly invasive green alga *Caulerpa taxifolia*, despite poorer resolution at the mitochondrial 16S locus.

In some cases, supplementation of phylogenetic analysis based on DNA sequence data with population genetic analysis of differentiation at multiple highly variable nuclear loci (e.g., microsatellites) can confirm or extend inferences of cryptic diversity. Although of limited utility for investigating interspecific crypsis due to problems with cross-species

transferability, microsatellite loci can be powerful tools for recognizing cryptic multiple introductions. In one case, [Saltonstall's \(2002\)](#) phylogeographic study of the common reed *Phragmites australis* based on DNA sequence data clearly revealed the presence of an invasive Eurasian lineage in the eastern United States, but also suggested the presence of a second invasive lineage in the southern United States. Later microsatellite analysis further supported this hypothesis, indicating high levels of differentiation between the southern US populations and all native *P. australis* ([Saltonstall 2003](#)).

Reciprocal monophyly requires the absence of intermediate sequences, while nonoverlapping geographic distributions of haplotypes require the absence of populations sharing those haplotypes. This suggests that sampling intensity, as noted above, can be a critical issue when inferring multiple evolutionary lineages, as such inference depends largely on gaps in the phylogeographic reconstruction. Whether or not these gaps might be filled by additional sampling is a matter that warrants careful consideration ([Muirhead et al. 2008](#)). At the same time, the sampling intensity required to enable confident inference depends largely on the degree of phylogeographic structure in the native range ([Taylor & Keller 2007](#)). Taxa with dramatic geographic structuring of genetic diversity may require only moderate sampling in order to infer cryptic lineages in the introduced range; in contrast, for some species with poor native phylogeographic resolution, there may be no level of sampling effort that allows inference of cryptic multiple invasions with high confidence ([Darling et al. 2008](#)). This problem will only be exacerbated by anthropogenic shuffling of native genetic diversity. The difficulties associated with cryptic invasions thus mirror those associated with source tracking (see Section IV, below).

How does one determine if cryptic diversity should be attributed to the presence of multiple cryptic species? While there are useful heuristics, there is no operational means to distinguish between inter- and intraspecific differentiation using strictly genetic criteria. In practice, researchers have relied primarily on phylogenetic pattern and the degree of genetic differentiation between samples. Specifically, reciprocal monophyly is often taken as an indication of independent species status. However, this rule should be applied with caution. [Meusnier et al. \(2002\)](#), for example, suggests the possibility of cryptic sibling species in *Caulerpa taxifolia*, based in large part on reciprocal monophyly between two invasive clades. Given the moderate levels of divergence between these clades, those authors wisely

qualify this claim by noting that levels of genetic differentiation observed may simply reflect intraspecific divergence. Similarly, [Zardus & Hadfield \(2005\)](#), despite observing reciprocal monophyly between clades of the invasive barnacle *Chthamalus proteus* in the Hawaiian Islands, decline to attribute **cryptic species** status to these clades as the genetic distance between them was considerably less than previously observed interspecific distances in the same genus. Such phylogenetically relevant comparisons may provide some measure of the interspecific divergence to be expected at a locus, but should also be interpreted cautiously and with consideration for ecological differences that may influence rates of speciation ([Folino-Rorem et al. 2009](#)). In some extreme cases, the sheer magnitude of the observed genetic distance between samples may be sufficient to suggest cryptic species. For instance, [Holland et al. \(2004\)](#) observed genetic distances at the COI locus of over 20% between samples previously attributed to the invasive jellyfish species *Cassiopea andromeda*, consistent with a time since the most recent common ancestor shared by “intraspecific” clades of up to 40 million years. For a single biological species to exhibit such variation would be truly remarkable; indeed, thorough sampling of the Indo-Pacific distribution of *Cassiopea* reveals numerous phylogenetic breaks indicative of a cryptic species complex ([Holland et al. 2004](#)).

The lack of an operational definition of inter- vs intraspecific genetic diversity strongly recommends adoption of integrated taxonomic approaches to the “discovery” of cryptic invasive diversity at the species level. Most authors accordingly offer cautious and preliminary suggestions for the existence of novel species. In some cases of marine invasions, additional support for these suggestions is available in the form of ecological differentiation between genetic lineages (see above) or biogeographic evidence suggesting reproductive isolation ([Andreakis et al. 2007](#)). In only two instances has phylogeographic inference of cryptic marine lineages been coupled with what many would consider the final arbiter of good biological species: experimental cross-breeding. [Caputi et al. \(2007\)](#) demonstrated reproductive isolation between cryptic species of *Ciona intestinalis*, aided not a little by the fact that this taxon is an established model organism amenable to reciprocal fertilization studies in the laboratory. In contrast, lineages of *Polysiphonia harveyi* appear to be completely interfertile despite reciprocal monophyly and divergence levels commensurate with interspecific differentiation in related taxa ([Mcivor et al.](#)

[2001](#)).^{[[Reviewer comment: Not sure if you want to briefly mention the fact that taxonomists themselves disagree about what constitutes a species--the phylogenetic species concept would largely focus on diagnostic characters rather than ability or inability to interbreed.]]}

IV. BIOGEOGRAPHY: THE ORIGINS OF INVASIVE SPECIES

^{[[AU: AR House Style calls for intervening text between headings. Pls. supply a paragraph here to introduce the following subsections.]]}

A. Why Do We Want to Know?

Contemporary recognition of the scale of recent biological invasions has led to the belated realization that the study of marine biogeography has largely neglected the role of humans in shaping the distributions of marine species ([Carlton 2003](#)). Indeed, some have semiseriously termed the modern geological period as the “Homogocene” ([Didham et al. 2005](#)) to draw attention to the accelerated pace of biotic mixing. All accounts of “cosmopolitan” species or those with peculiarly disjunct distribution must now be viewed critically, with the hypothesis of biological invasion requiring refutation. If native status is rejected, the true natural distributions often remain unknown. Thus, one rationale for discovering the origins of invasive species is to recover pre-Homogocene patterns of species distribution that are the primary data for the study of biogeography.

There are more pragmatic reasons to identify sources for specific introduced species. Knowledge of sources can help to define the ecological characteristics of each species and predict the extent of ultimate distribution in the region of introduction ([Kolar & Lodge 2001](#)). [Carlton & Cohen \(2003\)](#), for example, made explicit predictions of the spread of the crab *Carcinus maenas* based on its range in Europe, helped by the large body of ecophysiological literature that exists for this species. Synecological study in native regions can also suggest the extent to which predators, competitors, parasites, and pathogens limit a species distribution or regulate its populations. Such information can contribute to predicting the expansion of introduced species and possibly suggest avenues for biological control.

Finally, knowledge of sources helps to identify routes and vectors, suggesting options to reduce further invasions. [Kado \(2003\)](#) described populations of the northeastern Pacific

barnacle *Balanus glandula* in Ofunute Bay on Hokkaido Island, Japan, and suggested that possible vectors could have been ships from the Klawak, Alaska or the Puget Sound, Washington ports, both with significant shipping traffic to Ofunute Bay. [Geller et al. \(2008\)](#) subsequently demonstrated by genetic analysis of native and Japanese populations that central and southern California were improbable sources while a source in Alaska or Puget Sound was consistent with the genetic data.

B. How Do We Determine Sources?

As discussed earlier, many species are cryptogenic, and by definition we do not know the sources of those that may be nonnative. Criteria exist for refuting native status; however, determining the status of a local population as nonnative does not necessarily point to any particular native source. In other cases where taxonomy is well worked out, we may confidently know that a species has been introduced. In this case, we will know the general native distribution but not the exact source population. For the purposes outlined above, this is not especially satisfying. We expect that many or most species contain genetic, physiological, and ecological variation that may be relevant to ecological impacts or response strategies, and therefore more specific location of the source of an introduced species is highly desirable. As noted earlier, the identification of source regions shares much with the topic of **cryptic invasions**, for both involve the use of existing genetic variation. For **cryptic species** invasions, uncovering this variation is necessary to reveal species distinctions. For distinguishing sources of a single introduction, revealing native variation allows us to assign likelihood (in a qualitative or quantitative way) of different potential sources.

There are several preconditions for the genetic identification of a source, and the most important is the existence of significant geographic structure to genetic variation in the native range of the studied species. **Figure 2** illustrates the relationship between genetic structure in native populations and the resolution possible for identification of a source by genetic data. If native populations are genetically homogeneous due either to lack of genetic variation in the chosen loci or to high levels of gene flow throughout the species' range, it will be impossible to pinpoint a source at any useful geographic scale. On the other hand, if native genetic variation is strongly partitioned at very fine scales (i.e., local populations are highly differentiated from each other), it should be theoretically possible to precisely

determine the source of an introduction. Unfortunately, in practice, limits to sampling of native populations will often miss the true source in this case, and genetic methods will be unable to determine any likely source at all. Where moderate genetic structure exists, for example, in the form of isolation by distance, it is theoretically possible to identify regions within a species' range that are more plausible sources than others. In many marine species there exist strong genetic discontinuities that separate populations into relatively homogeneous, distinct groups. These discontinuities may be extremely abrupt, in the form of a phylogeographic break, or spread over a short distance as a genetic cline. In these cases, the regional source of an invasion may be readily determined as lying on one side or the other of a genetic breakpoint, but further precision may not be achievable.

<COMP: PLEASE INSERT FIGURE 2 HERE>

Figure 2 Conceptual relationships between the spatial scale of genetic structure of native populations and the probability that a source of an invasion can be identified, the ease of adequate sampling as a function of native genetic structure, and the impact of genetic drift on resolution of sources. Introduced species with fine-scale native genetic structure theoretically can be sourced with very high precision, but the likelihood of sampling the actual source population is low. In such cases, introduced samples will rarely show genetic affinities for any of the sampled native populations. Further, precision in these cases is most impacted by founder effects or genetic drift; immediately following the invasion event ($t = 0$), precision may be high, but it will degrade over time ($t = n$). Introduced species without much native genetic structure cannot be traced with any reasonable precision to a source within the native region, despite the ease of sampling genetic diversity adequately. Species with moderate genetic structure are thus the best candidates for successful genetic analysis. It should be noted that the spatial scale of genetic structure is a function not only of the taxon being investigated but of the genetic locus used in the investigation.

Another important precondition is that processes such as genetic bottlenecking, drift, and selection have not caused introduced (or source) populations to have greatly diverged since the time of invasion. Even if native populations are genetically subdivided, the colonists that comprise founding invading populations often contain a small sample of the total genetic diversity in their source population ([Dlugosch & Parker 2008](#), [Roman & Darling 2007](#)). This genetic bottleneck may purge the introduced population of less common haplotypes that associate it with its true source and increase the number of equally probable source populations. Similarly, if founding populations are small, genetic drift (especially for older invasions) will cause further erosion of genetic diversity and again increase the number of genetically plausible sources. Frustratingly, bottlenecks and drift decrease resolution of

sources only for those taxa that were good candidates for inference of source in the first place---those with strong native genetic structure. A set of large, interconnected native populations may be less likely to drift, but an introduced subsample of one such population may show no genetic affinity for any particular native locale. Thus poor resolution simply remains poor.

It is also important to recognize the potential for temporal variation in both invasive and native populations and its possible impact on inference of origins. Invasions are dynamic processes, and the distribution of genetic variation can be expected to change, sometimes dramatically, throughout the course of invasion. Many standard population genetics studies disregard such changes, and may even make the assumption (often out of necessity imposed by limited collection resources) that temporal variation can safely be ignored when comparing spatially separated samples collected in different time periods ([Depaulis et al. 2009](#)). This assumption should be viewed with suspicion when dealing with invasive populations, and temporal sampling may reveal dynamic properties of population expansion that would be impossible to determine using standard sampling approaches ([Selkoe et al. 2008](#)). For instance, Hoos (X.X. Hoos, unpublished paper[**AU: I have formatted this per house style; pls. include the first initials of the author**]), by investigating both contemporary and museum-held samples of the Atlantic gem clam *Gemma gemma*, has shown that mitochondrial haplotype frequencies in both the native and invasive ranges of this species have changed dramatically since the late nineteenth century. Such temporal fluctuations in genetic structure force the conclusion that our knowledge of haplotype frequencies at the time of introduction is too poor to accurately pinpoint native sources. It is worth noting two factors likely to mediate changes in genetic structure in native populations. First, species with strong native population subdivision are more likely to be affected by drift, as subdivision arises through limited exchange among small (relative to the total population) subpopulations. Second, human disturbance of habitats in native and nonnative regions may contribute to fluctuations in metapopulation structure (local extinctions, recolonizations, dramatic demographic changes within subpopulations) that could dramatically affect population genetic structure. Unfortunately, invasive marine species are in many cases precisely those most likely to be influenced by such disturbance,

and the impacts will only become stronger as the time between introduction and genetic sampling increases.

Although a number of studies have sought to identify sources of marine invasions, it is rarely possible to confirm the accuracy of these attempts. An ideal test case would involve well-documented intentional introductions followed by genetic analysis after known time intervals or generations. In a nonmarine example, [Eldridge et al. \(2001\)](#) used microsatellite data and a Bayesian assignment test to correctly identify the source population of transplanted rock wallabies. However, in this case the time lag was short and the tested individuals were the actual transplanted animals or F1 offspring. Although documented introductions have been used to study genetic consequences of invasion, we found few attempts to use historically documented introductions to test methods of assigning sources. The transplanting of the American oyster, *Crassostrea virginica*, to the western United States is one case where documentation is strong ([Barrett 1963](#), [Carlton 1979a](#), [Miller et al. 2007](#)) and could serve as a useful test platform for genetic analyses. While populations of *C. virginica* could not be established in the western United States, scores of oyster-associated species were cointroduced and persist today. Recent study of one such species (the aforementioned *Gemma gemma*) suggests that while historical documentation may indeed provide means to assess accuracy of source assignments, in many cases elucidation of complex patterns of sources and routes may be necessary to make such documentation compatible with available genetic data (X.X. Hoos, unpublished paper[**AU: I have formatted this per house style; pls. include the first initials of the author**]).

In general, the integration of nongenetic data can greatly strengthen inferences regarding sources of invasive populations and the mechanisms by which they have expanded their ranges. Most genetic studies only loosely incorporate nongenetic data, typically using limited historical documentation to corroborate genetic inference of sources. However, a growing number of researchers are adopting analytical methods that more directly integrate genetic and nongenetic data. [Herborg et al. \(2007\)](#), for example, examined the correlation between shipping patterns and population genetic structure of the invasive Chinese mitten crab *Eriocheir sinensis* to infer a role for continued ballast water transport in spread of the species throughout Europe. Similarly, [Dawson et al. \(2005\)](#) integrated genetic analysis with a global biophysical model of potential natural dispersal to confirm multiple introduction of

the moon jelly *Aurelia* in the Pacific. Hopefully, such work presages future developments that further enhance the utility of genetic analysis for understanding marine bioinvasions.

C. Analytical Methods

The most commonly used method to identify a source is through identification of haplotypes unique to different regions within the putative source. Such “private” haplotypes can be used to infer a source by matching haplotypes in introduced and source populations. At one extreme, private alleles are present in native populations at low frequency and arise as recent mutations in genetic loci after populations are isolated, or conversely, by sorting of alleles during isolation of populations. In these cases, private alleles are genealogically related to alleles shared among populations. This is relevant because phylogenetic analysis will not be informative and conclusions will depend on population genetic processes (which may not be well understood), as well as the adequacy of sampling (see below). At the other extreme, populations have achieved **reciprocal monophyly** for sampled genetic loci through prolonged genetic isolation: This corresponds to the phylogeographic break described above. In such cases, identification of the source region is straightforward, although resolution may be poor.

Another commonly used approach is to make pairwise comparisons of genetic distance based on proportions of shared alleles or nucleotide divergence (**Figure 3A**). This approach assumes that an introduced population will be most similar to the population from which it was drawn. Clearly, this assumption is not always true, as in the case where potential source populations are undifferentiated. In addition, as stressed above, introduced populations may have lost genetic diversity through founder effects, and continuing genetic drift will cause dissimilarity of source and founding populations. A related approach is pairwise comparisons of F_{ST} or its nucleotide analog Φ_{ST} , both measures of population subdivision. In populations at equilibrium, smaller F_{ST} or Φ_{ST} values indicate a high degree of connectivity (through gene flow). However, recently introduced populations are not at genetic equilibrium, and when a vector for introduction has closed, there is no connectivity between native and invasive populations. Consequently, F_{ST} can be considered an alternative measure of genetic distance, but no inferences about population genetic process should be made.

<COMP: PLEASE INSERT FIGURE 3 HERE>

Figure 3 Two methods for determining sources of introduced populations. The example is drawn from study of the globally invasive European green crab *Carcinus maenas* (adapted from [Darling et al. 2008](#)). (a) Neighbor-joining (NJ) tree based on microsatellite genetic distances between populations; [Reviewer inserts: presumed invasive populations in bold]. Well-supported groups in the NJ tree include native *C. maenas* (1, [**Reviewer inserts: European localities,**] along with two introduced populations in Cape Town [**Reviewer comment: (CAP??)**] and Nova Scotia [**Reviewer comment: (MUR????)**]), native *C. aestuarii* (2, [**Reviewer comment: from ???**]), and introduced populations in Japan (3), eastern and western United States (4), and Australia and Argentina (5). Groupings suggest that both the western U.S. invasion (RED, TOM, WIL) and the Argentine invasion (ARG) source to earlier invasive populations in the eastern United States (MYS, BAR) and Australia (COR, FAL), respectively. (b) Assignment of individual multilocus microsatellite genotypes based on the Bayesian clustering method implemented in the program STRUCTURE. Individual genotypes are represented by narrow vertical lines; proportional ancestry in five predicted clusters is indicated by color. As in (a), note that the western U.S. populations cluster with the eastern United States, the Argentine population clusters with Australia, and the Nova Scotia population, though geographically proximate to the eastern United States, does not belong to the U.S. cluster (earlier analysis of this population indicated that it derives from an independent introduction to North America).**

The equilibrium assumptions of such summary statistics limit their utility for inferring genetic origins of introduced populations. Fortunately, alternatives that avoid these assumptions do exist ([Davies et al. 1999](#)). In particular, assignment methods have been frequently adopted by invasion geneticists. These methods generally attempt to assign individual genotypes to their most likely populations of origin by calculating the probability that a multilocus genotype could arise from a candidate source population given its estimated genotype frequencies ([Waser & Strobeck 1998](#)). Slightly different approaches have been implemented in software such as the widely used STRUCTURE ([Falush et al. 2003](#), [Pritchard et al. 2000](#)). Rather than assigning individuals to predefined potential sources, these programs cluster individual genotypes without a priori definition of populations; when native and introduced individuals are found in the same cluster, this can be interpreted as evidence for a source-invader relationship (**Figure 3b**). These are powerful approaches when samples are taken immediately subsequent to invasion---for example, by examining initially invading propagules. When vector-paths remain open, assignment tests might point to a specific source population. Such assignment of first-generation migrants might be of considerable importance in studying the spread of invasive species in nonnative regions ([Rollins et al. 2009](#)).

Applications to long-standing introduced populations may not be so straightforward. As the time interval widens between the founding of an introduced population and genetic sampling, assignment tests will suffer the same degradation of performance as other methods. Many assignment tests ([Piry et al. 2004](#), [Pritchard et al. 2000](#), [Rannala & Mountain 1997](#)) do not explicitly allow source or introduced populations to evolve, therefore the contemporary population that represents the true source region may have quite different genotype frequencies than at the time of invasion. It may be that, on average and given purely neutral processes, the true source will be the most likely source despite drift or selection. However, without genetic data at the time that the invasion occurred, we cannot know whether change is neutral or not. [Rannala & Mountain \(1997\)](#) voice confidence in their methods for only two generations following migration. Perhaps for these reasons, in a discussion of applications of assignment tests in ecology and evolution, invasions were not mentioned ([Manel et al. 2005](#)). [Davies et al. \(1999\)](#) did specifically include biological invasions as an application of assignment tests, but also cautioned that genetic drift may be important when invasions are not recent. Newer approaches do in fact model drift in isolated populations ([Hellenthal et al. 2008](#)). Other new approaches allow explicit migration and demographic scenarios to be tested against each other for plausibility ([Hellenthal et al. 2008](#), [Jobin & Mountain 2008](#)), an approach that might have excellent application to invasions when we wish to evaluate competing historical hypothesis. However, we reiterate the lesson that each new method requires a dose of skepticism while its efficacy is explored and tested with a variety of cases.

Geographic and temporal sampling adequacy is a persistent problem in the inference of sources. Temporal sampling is almost never done, and we have a very poor grasp of how important this can be. At an extreme, the nonneutral genotypes might vary over the span of months due to strong selection acting on settling cohorts, as in the case of *Mytilus* populations on Long Island, New York ([Koehn et al. 1980](#)). This would not be discerned without careful attention to the sizes (or better, ages) of sampled individuals. There is also a bias in geographic sampling intensity. In most cases, investigators are most interested in a locally introduced species and may sample invasive populations, which are geographically confined, exhaustively. Putative source regions, in contrast, are often distant from the researchers and potential source populations can include an entire biogeographic region.

Consequently, the native range of an introduced species is almost always inadequately sampled. Oftentimes, the distinction between the actual source and the most likely source among those sampled is not strongly made. In an introduced population of the Mediterranean and Iberian gastropod *Cyclope neritea* in the Bay of Biscay, [Bachelet et al. \(2004\)](#) observed mixtures of mitochondrial COI haplotypes that did not co-occur in two sampled native populations. Consequently, multiple independent introductions were inferred, justified by the deep divergence among the mixed haplotypes. Given the data, more than one introduction is a necessary conclusion. Yet, with only two native populations sampled, one on the coast of Portugal and one in the Mediterranean, the actual geographic distribution of genetic variation in this species is unknown. Also yet to be determined are the possibility of a genetically mixed population that could be a single source in the transition between basins, and the actual status of Atlantic and Mediterranean populations as a single species. The presence of haplotypes in the introduced population not found in the two sampled native populations also suggests that the native range is undersampled.

Sampling strategies in the nonnative range deserve similar attention. Models of invasive spread now generally acknowledge that mechanisms driving that spread may be influenced strongly by spatial scale ([Hastings et al. 2005](#), [Pauchard & Shea 2006](#)). The possibility that different mechanisms of spread may be operative on different spatial scales underscores the importance of spatially stratified sampling of introduced populations ([Ward 2006](#)). Some recent studies of invasive marine taxa have already revealed the utility of this approach for uncovering scale-dependency of population genetic structure attributable to differences in the efficacy of various dispersal mechanisms at different scales ([Dupont et al. 2009](#), [Viard et al. 2006](#)). The observation of secondary introductions (introductions sourced to populations that are, themselves, introduced) in marine bioinvasions ([Darling et al. 2008](#)) draws attention to the fact that not all sources are native. Thus in some cases, consideration of genetic structure over multiple spatial scales may be necessary to capture all salient aspects of invasion history[**Reviewer comment: For a livelier ending, why not return to the theme of the importance of invasions and perhaps briefly mention what is happening at the moment in the Caribbean with invasive lionfish?**].

DISCLOSURE STATEMENT

[AU: Please insert your Disclosure of Potential Bias statement, covering all authors, here. If you have nothing to disclose, please confirm that the statement below may be published in your review. Fill out and return the forms sent with your galleys, as manuscripts CANNOT be sent for pageproof layout until these forms are received.**]**

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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SUMMARY POINTS

1. Molecular genetics provide powerful tools for helping to clarify the provenance of cryptogenic species, alerting us to the possibility of cryptic invasions at multiple levels of the taxonomic hierarchy, and recognizing the geographic sources of introduced populations. The inferences made through these approaches have important implications for development of appropriate management strategies and scientifically defensible policies.
2. Genetic studies of invasive populations make us aware of substantial gaps in our systematic and biogeographic knowledge of marine taxa in their native ranges. This awareness is critical given the recognized inadequacy of our current understanding of marine biodiversity and the increasing anthropogenic stresses on marine ecosystems. Invasion genetics may thus provide important entrees into future clarifications of the taxonomy and geographic distribution of marine species.
3. Temporal and geographic sampling for genetic studies is almost always inadequate (particularly in the native range) and invasion biologists still occasionally lack caution in the interpretation of genetic data. In most cases, inferences of cryptic invasions, multiple introductions, and sources of invasive populations should be offered as hypotheses warranting further exploration. The strongest support for such hypotheses will often come in the form of parsimonious reasoning backed by independent (i.e., nongenetic) modes of inference.

4. The peculiar genetics of recent introductions violates the equilibrium assumptions of many standard genetic approaches and requires special attention to the appropriate choice of genetic loci and use of statistical tools for data analysis. Limitations imposed by biogeographic structure (or lack thereof) in the native range can be overcome in part by selection of appropriate genetic loci, underlining the importance of multilocus approaches. For some applications, such as assignment testing to determine native sources, the availability of multilocus datasets (e.g., microsatellites, AFLP, SNPs, etc.) is absolutely crucial.

FUTURE ISSUES

1. Future developments in statistical analysis of genetic data may substantially improve our understanding of marine bioinvasions. For instance, limitations to current assignment-testing methods may prevent accurate assignments of introduced populations that have experienced strong founder effects and drifted substantially from their sources. Explicit incorporation of drift into these methods could significantly enhance their utility to invasion biologists. Similarly, statistical approaches that allow competing hypotheses to be explicitly tested, or that allow integration of genetic data with nongenetic information relevant to invasion history (e.g., strength of vectors) could prove extremely useful.
2. The aims of invasion biology and DNA barcoding overlap substantially, and this overlap should be exploited to support important research goals. While genetic study of marine bioinvasions continues to provide examples of previously undescribed diversity (one of the explicitly stated objectives of barcoding), the systematic population of reference databases with genetic information on well-described marine taxa would greatly facilitate the future study of marine bioinvasions.
3. Discovery of cryptic invasions, particularly at the species level, should be recognized as an opportunity to initiate integrative taxonomic revisions of marine flora and fauna. Genetic methods may be the most likely tools by which such cryptic diversity is first detected, but genetics alone cannot determine the systematic relationships between novel and previously described biodiversity, nor can it uncover the important ecological correlates of that diversity.

4. International collaborations provide perhaps the strongest mechanism to sample native diversity adequately. Unfortunately, limits to interdisciplinary communication, funding, and even interest may prevent researchers from moving to mend these gaps. It may be difficult for invasion biologists studying a critically important invasive taxon to encourage collaboration with ecologists and biogeographers in the native range who may consider the same taxon relatively unimportant or uninteresting. Given the global nature of the problem, researchers and funding agencies alike should recognize these opportunities not only to improve our understanding of marine invasions, but also to substantially reshape our knowledge of biodiversity and biogeography in the world's oceans.

Sidebar: Recognizing Invasions

A number of nonmolecular criteria exist for recognition of introduced species. Both historical and modern biogeography may reveal the appearance of a species in a region where it was not previously known, based upon (as applicable) paleontological, archeological, and historical evidence. In addition, highly disjunct distributions (e.g., a species is broadly distributed in one ocean but exists as isolated populations in other oceans) are strongly suggestive of introduced status. Vector biology can highlight species known to be capable of, or directly associated with, human-mediated transport such as ballast water---or, on the other hand, known to be incapable of being transported by nonhuman mechanisms such as open ocean currents (as larvae or on drifting materials). Ecological relationships may implicate species restricted primarily to human-created, disturbed habitats in the region where the species is thought to be nonnative (i.e., weeds live with weeds). Potential introductions may also include symbionts or predators exclusively associated with known nonnative species. Finally, evolutionary biology may lead us to suspect a history of introduction for a taxon when its most closely related morphological species, or species group, occurs in another ocean or on another continent.

<NOTE>COMP: [END SIDEBAR] </NOTE>

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Table 1 The varieties of “cryptic invasions” categorized by two criteria: the novelty of the observed diversity (whether or not it has been previously described), and the taxonomic level of that diversity

Novelty of described diversity[**Reviewer comment: I do not get the significance of distinguishing between columns 2 and 3. The distinction being made needs clarification.**]		
Taxonomic level of diversity	Diversity previously described	Discovery of novel diversity
Inter-specific diversity (e.g., cryptic species invasions)	A) cryptic species invades but is mistaken for native species	C) Two or more cryptic species invade but are mistaken for a single morphospecies
	B) multiple cryptic species invade but are mistaken for a single invasive species	D) cryptic species invades; later shown to be one of multiple species previously thought to be a single cosmopolitan morphospecies
Intra-specific diversity (e.g., multiple introductions)	E) cryptic clade invades but is mistaken for an already established clade of the same species	F) cryptic clade invades but is mistaken for native clade of the same species
		G) multiple cryptic clades of the same species invade

Table 2 Genetic studies of cryptic invasions in marine ecosystems[**Reviewer comment:
Perhaps provide phylum or common name before species name?**]

Category ^a	Taxon	Native range	Introduced range	Genetic locus/loci	Reference(s)
Cryptic species					
A	<i>Mytilus</i>	Mediterranean	northeastern Pacific	mtDNA (16S), allozymes	Geller et al. 1994 , McDonald & Koehn 1988
A	<i>Antarctothoa</i>	Southern hemisphere	Spain	mtDNA (COI)	Hughes et al. 2008
A	<i>Polysiphonia</i>	Japan	New Zealand	cpDNA (<i>rbcL</i>)	McIvor et al. 2001
A/F	<i>Ophiactis</i>	Indo-Pacific	western Atlantic	mtDNA (COI)	Roy & Sponer 2002
B	<i>Carcinus</i>	Europe/Mediterranean	South Africa, Japan	mtDNA (16S)	Geller et al. 1997
C	<i>Atherinomorus</i>	Red Sea	Mediterranean	mtDNA (control region)	Bucciarelli et al. 2002
C	<i>Undaria</i>	Japan	various	mtDNA (intergenic spacers)	Voisin et al. 2005
C	<i>Marenzelleria</i>	northwestern Atlantic	Baltic Sea, North Sea	mtDNA (16S)	Bastrop et al. 1998
C	<i>Asparagopsis</i>	various	various	nDNA (28S), cpDNA (<i>rbcL</i>), mtDNA (intergenic spacer)	Andreakis et al. 2007
C	<i>Gracilaria</i>	western Pacific	western Atlantic	mtDNA (<i>cox2-3</i> spacer)	Thomsen et al. 2006
C	<i>Watersipora</i>	various	various	mtDNA (COI)	Mackie et al.

C	<i>Carijoa</i>	various	various	nDNA (SRP)	2006 Concepcion et al. 2008
C	<i>Bugula</i>	northwestern Atlantic	northeastern Pacific	mtDNA (COI)	Davidson & Haygood 1999
C	<i>Cercaria</i>	Japan	northeastern Pacific	mtDNA (COI)	Miura et al. 2006
C	<i>Cordylophora</i>	Ponto-Caspian	various	nDNA (28S), mtDNA (16S, COI)	Folino-Rorem et al. 2009
C	<i>Cassiopea</i>	various	Hawaii	mtDNA (COI)	Holland et al. 2004
D	<i>Aurelia</i>	various	various	mtDNA (COI)	Dawson et al. 2005
D	<i>Ciona</i>	Europe	various	nDNA (ITS, <i>Hox</i> introns/exons), mtDNA (COI), microsatellites	Caputi et al. 2007
Multiple introductions					
E	<i>Carcinus maenas</i>	Europe	northwestern Atlantic	mtDNA (COI), microsatellites	Roman 2006, Darling et al. 2008
F	<i>Phragmites australis</i>	Eurasia	eastern and southern United States	cpDNA (noncoding), microsatellites	Saltonstall 2002, 2003
G	<i>Cyclope neritea</i>	Iberia	France	mtDNA (COI)	Simon-Bouhet et al. 2006
G	<i>Polysiphonia harveyi</i>	Japan	Europe, New Zealand	cpDNA (<i>rbcL</i>)	McIvor et al. 2001
G	<i>Codium fragile</i>	Japan	various	cpDNA, cp microsatellites	Provan et al. 2005
G	<i>Caulerpa taxifolia</i>	Australia	Mediterranean	nDNA (ITS), cpDNA (16S intron)	Meusnier et al. 2002
G	<i>Asparagopsis armata</i>	Indo-Pacific, Atlantic	Mediterranean	nDNA (28S), cpDNA (<i>rbcL</i>), mtDNA (intergenic spacer)	Andreakis et al. 2007
G	<i>Chthamalus</i>	Caribbean	Pacific	mtDNA (COI)	Zardus &

	<i>proteus</i>	eastern/western Atlantic			Hadfield 2005
G	<i>Undaria pinnatifida</i>	Japan	New Zealand	mtDNA (cox3), microsatellites	Uwai et al. 2006 , Voisin et al. 2005 , Daguin et al. 2005
G	<i>Ocinebrellus inornatus</i>	western Pacific	northeastern Pacific, France	mtDNA (COI, 12S, 16S), allozymes	Martel et al. 2004
G	<i>Marenzelleria neglecta</i>	northwestern Atlantic	Baltic Sea, North Sea	mtDNA (16S, COI, cytb)	Bastrop & Blank 2006 , Bastrop et al. 1998
G	<i>Caprella mutica</i>	western Pacific	northeastern Pacific, north Atlantic	mtDNA (COI)	Ashton et al. 2008
G	<i>Asparagopsis taxiformis</i>	Indo-Pacific	Mediterranean	microsatellites	Andreakis et al. 2009
G	<i>Gammarus tigrinus</i>	northwestern Atlantic	Europe	mtDNA (COI)	Kelly et al. 2006b
G	<i>Loxathylacus panopaei</i>	Gulf of Mexico	northwestern Atlantic	mtDNA (COI)	Kruse & Hare 2007
G	<i>Botryllus schlosseri</i>	Atlantic Europe	Mediterranean	mtDNA (COI)	Lopez-Legentil et al. 2006
G	<i>Spartina alterniflora</i>	northwestern Atlantic	northeastern Pacific	cpDNA, microsatellites	Blum et al. 2007

^aCorresponds to categories in [Table 1](#).

<NOTE>COMP: DO NOT SET THE FOLLOWING LIST AS SUCH. IT WILL BE USED BY OUR ELECTRONIC CONTENT COORDINATORS IN THE ONLINE VERSION OF THE ARTICLE.</NOTE>

Terms and Definitions

Cryptic species: a single morphospecies comprising one or more biological species (genetically distinct and theoretically/demonstrably reproductively isolated)

Cryptic clades: morphologically indistinguishable monophyletic lineages embedded within a single biological species

Cryptic invasion: an invasion that remains unrecognized because the invader is mistaken for native or previously introduced cryptic species or clades

Multiple introductions: introductions of a single biological species to a single recipient region from two or more genetically distinct sources

Cryptogenic species: a species for which the evidence is insufficient to determine whether it is native or introduced

Reciprocal monophyly: in a phylogeny, when all members of a lineage share a more recent common ancestor with each other than with any other lineage

Morphospecies: a species defined entirely on the basis of morphological characteristics