

Adaptation in marine invasion: a genetic perspective

C. K. Tepolt

Biological Invasions

ISSN 1387-3547

Volume 17

Number 3

Biol Invasions (2015) 17:887-903

DOI 10.1007/s10530-014-0825-8



Your article is protected by copyright and all rights are held exclusively by Springer International Publishing Switzerland. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".

Adaptation in marine invasion: a genetic perspective

C. K. Tepolt

Received: 30 December 2013 / Accepted: 9 December 2014 / Published online: 16 December 2014
© Springer International Publishing Switzerland 2014

Abstract Genetic adaptation—heritable changes that alter an organism's performance—may facilitate invasion at several scales, but is seldom considered in predicting and managing marine invasions. However, a growing body of research—largely based on emerging genetic approaches—suggests that adaptation is possible and potentially widespread in the marine realm. Here, I review evidence for adaptation in marine invasion, considering both quantitative and genetic studies. Quantitative studies, which consider trait-based differences between populations or individuals without directly examining genetic makeup, have suggested local adaptation in several high-profile species. This implies that invasion risk may not be constant from population to population within a species, a key assumption of most invasion models. However, in many quantitative studies, the effects of heritable adaptive changes may be confounded with the effects of plasticity. Molecular approaches can help disentangle these effects, and studies at the genomic level are beginning to elucidate the specific genetic patterns and pathways underlying adaptation

in invasion. While studies at this scale are currently rare in the marine invasion literature, they are likely to become increasingly prevalent—and useful—now that next-generation sequencing approaches have become tractable in non-model systems. Both traditional and emerging genetic approaches can improve our understanding of adaptation in marine invasions, and can aid managers in making accurate predictions of invasion spread and risk.

Keywords Adaptation · Genetics · Marine invasion · Next-generation sequencing · Selection

Introduction

Understanding how invasive species are able to survive and thrive in novel environments is vital to predicting the spread and persistence of species invasions, a key element of conservation planning and management (Hastings et al. 2005; Hewitt and Campbell 2007). This is especially important as global climate change continues, particularly in aquatic systems: in contrast to terrestrial species, aquatic invasive species may be more resilient to climate change than their native counterparts (Sorte et al. 2013). While resilience can take many forms, one particularly understudied mechanism in the marine realm is genetic adaptation—that is, heritable genetic change underlying a trait that influences organismal

C. K. Tepolt (✉)
Hopkins Marine Station of Stanford University, 120
Ocean View Boulevard, Pacific Grove, CA 93950, USA
e-mail: carolyn.tepolt@gmail.com

Present Address:
C. K. Tepolt
Smithsonian Environmental Research Center, 647
Contees Wharf Road, Edgewater, MD 21037, USA

fitness. However, there is an increasing body of evidence suggesting that this type of adaptation does play a significant role in the sea (Sanford and Kelly 2011). Molecular techniques have long played a role in investigating adaptation in marine systems (e.g. Koehn et al. 1980), and as these methods become more sophisticated, they are able to uncover subtle evidence of selection in an increasing number of species (Nielsen et al. 2009a; Pespeni and Palumbi 2013). By understanding the role of genetic adaptation in shaping marine invasion potential, we can improve predictions of invasion spread (Kearney and Porter 2009), allowing management to be based on more realistic models of how invasion risk may change.

Correlative environmental niche modeling (ENM) is commonly used for predicting invasive spread, and is based on the idea that the environmental conditions found in a species' native range can be used to predict its potential spread (Elith and Leathwick 2009; Jiménez-Valverde et al. 2011). However, a handful of recent terrestrial examples have shown that correlative ENM may fail to predict significant range expansions by invasive species that have adapted beyond their previous limits (Hill et al. 2013; Morey et al. 2013). In the marine realm, models based primarily on native range temperature failed to predict the spread of the European green crab *Carcinus maenas* beyond southern Nova Scotia in the east coast of North America (Compton et al. 2010). However, the species is thriving throughout the Maritimes and Newfoundland, in colder waters than predicted by the native range model (Blakeslee et al. 2010). In addition, models built for two genetically distinct native populations of this species predicted very different potential spread (Darling et al. 2008; Compton et al. 2010).

The failures of ENM to fully predict the invasive range of these species may be due in part to the fact that these predictions tend to treat a species as a single homogeneous entity, with a fixed and universal physiology (Deutsch et al. 2008). However, this is not the case: individual populations within a species may vary considerably in their environmental tolerance and plasticity (O'Neill et al. 2008; Preisser et al. 2008; Kuo and Sanford 2009; Sgrò et al. 2010; Sorte et al. 2011), and may have the capacity to adapt in response to environmental changes (Balanyá et al. 2006; Van Doorslaer et al. 2009). Researchers have begun to acknowledge that these factors are central to

predictions about the future of ecosystems, but very few empirical data are available to fill the gap between that ideal and the current one-size-fits-all approach (Pertoldi and Bach 2007; Atkins and Travis 2010; Chown et al. 2010; Hamann and Aitken 2013).

There is a growing body of quantitative research detailing physiological or morphological differences between populations of marine invasive species (Table 1). Quantitative studies consider trait-based differences between populations or individuals without directly examining genetic makeup. One shortcoming of many such studies is that it may be difficult to determine if these differences are truly heritable, or are the result of developmental or acclimatory plasticity (West-Eberhard 2003). In the case of some marine invasions, there is also the possibility that putatively population-level differences may instead be due to differences between uncharacterized cryptic species (Kruse et al. 2011; Mackie et al. 2012). In addition, experimental measures of differentiation may not have the resolution to detect subtle differences between populations, or may not measure the particular trait on which selection is acting.

In cases such as these, molecular tools may be extraordinarily useful for disentangling genetic adaptation from other influences, and for gaining a deeper understanding of the genetic pathways underlying adaptive differences. Importantly, molecular tools can be applied even to non-model species for which careful, multi-generational breeding programs are impossible. By using molecular tools, researchers can estimate how much variation is heritable, which aids in evaluating the appropriateness of climate envelope models (e.g. can you assume each population has the same tolerances as all populations collectively?). Genetics approaches can also elucidate the dynamics of adaptation: How many genes are involved? How quickly can an adaptive response arise? How important is standing diversity to adaptive potential? This information may help researchers understand and predict how invasive species may respond to future perturbations such as climate change. On a broader scale, invasions can be used to understand species' capacity to respond to climate change. Invasive species, by definition, are very good at exploiting novel environments, and thus provide a baseline for studying the traits that may facilitate success in a rapidly changing environment (Chown et al. 2007; Blackburn 2008; Sorte et al. 2010).

Table 1 Quantitative studies suggesting adaptation in marine invasive species

Species	PLD	Range	Factor	Trait or fitness proxy	Reference
<i>Carcinus maenas</i>	4–8 weeks	NI	Temperature	Heart failure, heart rate	Tepolt and Somero (2014)
<i>Littorina littorea</i>	4–7 weeks	I	Temperature	Survival, acclimation ability	Sorte et al. (2011)
<i>Crassostrea virginica</i>	2–3 weeks	N	Temperature	Growth, ciliary activity	Dittman (1997), Dittman et al. (1998)
<i>Venerupis philippinarum</i>	2 weeks	I	Pollution	Detoxification mechanisms	Paul-Pont et al. (2010)
<i>Venerupis philippinarum</i>	2 weeks	I	Parasitism	Infection	Paul-Pont et al. (2010)
<i>Amphibalanus amphitrite</i>	1–4 weeks	I	Pollution	Survival	Romano et al. (2010)
<i>Rhithropanopaeus harrisi</i>	1–3 weeks	NI	Temperature Salinity	Survival, development time, larval weight	Laughlin and French (1989)
<i>Eurytemora affinis</i>	1–3 weeks	NI	Salinity	Ion-motive enzyme activity, hemolymph osmolality	Lee et al. (2011, 2012)
<i>Acartia tonsa</i>	1 week	N	Hypoxia	Habitat selection	Decker et al. (2003)
<i>Watersipora subtorquata</i>	1 day	I	Pollution	Survival, growth	McKenzie et al. (2011, 2012)
<i>Styela plicata</i>	1 day	I	Pollution	Hatching success	Galletly et al. (2007)
<i>Botrylloides violaceus</i>	None	I	Temperature	Survival	Sorte et al. (2011)
<i>Botrylloides</i> spp.	None	I	Temperature	Survival, growth	Grosholz (2001)
<i>Botryllus schlosseri</i>	None	I	Temperature	Survival	Sorte et al. (2011)
<i>Bugula neritina</i>	None	I	Temperature	Survival	Sorte et al. (2011)
<i>Bugula neritina</i>	None	I	Pollution	Survival, growth, settlement	Piola and Johnston (2006)
<i>Diplosoma listerianum</i>	None	I	Temperature	Survival	Sorte et al. (2011)
<i>Littorina saxatilis</i>	None	N	Temperature	Survival, acclimation ability, enzyme activity	Sokolova and Förtner (2001), Sorte et al. (2011)
<i>Littorina saxatilis</i>	None	N	Predation	Morphology	Grahame et al. (2006)
<i>Littorina saxatilis</i>	None	N	Pollution	Survival	Daka and Hawkins (2004)
<i>Littorina saxatilis</i>	None	N	Predation Disturbance	Survival, growth, morphology, habitat selection	Janson (1982, 1983), Johannesson and Johannesson (1996)
<i>Littorina saxatilis</i>	None	N	Predation Tidal height	Survival, growth	Johannesson et al. (1997), Rolán-Alvarez et al. (1997)
<i>Littorina saxatilis</i>	None	N	Temperature Tidal height	Growth, reproduction	Pardo and Johnson (2005)
<i>Asparagopsis armata</i>	Unclear	N	Light	Morphology	Monro and Poore (2009)

PLD pelagic larval duration, Range whether the study was carried out in the species' native (N) or invasive (I) range or both (NI)

In this review, I will discuss evidence for genetic adaptation in marine invasion, derived from both quantitative and genetic studies. I consider genetic adaptation in marine invasion in the context of a continuum with three rough divisions: adaptation promoting invasion, local adaptation, and adaptive potential. Adaptation promoting invasion encompasses adaptive differences between invasive and closely-related species, and may shed light on the “origin of invasiveness”. Local adaptation describes adaptive differences between populations in a species’ native range, and implies that some populations of the same species may differ in their ability to invade a given region. Adaptive potential (or ongoing adaptation) considers adaptive differences that arise as a consequence of invasion, either between or within invasive populations, or between native and invasive populations. Adaptive potential is particularly understudied in marine systems, but may be critical to determining a species’ future invasion potential, especially under climate change. Both local adaptation and adaptive potential are neglected in most models of invasion spread, and may significantly impact the performance of those models. I will describe in detail particularly good case studies for each aspect of adaptation in invasion, as well as the importance of linking molecular and quantitative measures of diversity. Finally, I will discuss the tremendous potential of next-generation sequencing approaches for studying genetic adaptation in marine invasion.

Evidence for genetic adaptation in marine invasion

Genetic adaptation requires heritable genetic change underlying a trait that influences organismal fitness. To date, there are very few studies in the marine invasion literature that demonstrate a clear link between variation in a trait and specific genetic mechanisms. Here, I discuss papers that suggest adaptation in widespread invasive marine species. As a general rule, I included species that were listed in the marine invasions database created by Molnar et al. (2008) and administered by The Nature Conservancy (available at <http://www.conservationgateway.org/ConservationPractices/Marine/Pages/marineinvasives.aspx>). In addition, I included a handful of species not listed in the database but with well-documented invasive status (e.g. *Watersipora subtorquata* and

Balanus glandula). For the purposes of this review, I have included only those species that have a definite invasive range (i.e. are not fully cryptogenic) and that have fully marine or brackish life cycles. The last consideration meant that no fish were included in this review, as the only adaptive studies I am aware of in invasive fish concern anadromous or freshwater species (reviewed in Conover et al. 2006).

There is a considerable body of indirect evidence for genetic adaptation in invasion (Tables 1, 2). Some of these studies are correlative and speculative, as when the trait of interest varies between populations under presumably different selective pressures (Sorte et al. 2011). While this evidence is suggestive, without multi-generational common garden rearing it is impossible to definitively attribute these differences to genetic adaptation as opposed to plasticity. Other quantitative studies take advantage of the clonal nature of many fouling organisms to examine variation between individuals in an implicitly genetic context, providing clearer evidence of molecular evolution (McKenzie et al. 2011, 2012).

Adaptation between congeners also plays an important role in marine invasion (Table 2). Molecular tools often reveal that apparently widespread invasive species are instead cryptic species complexes, where genetic lineage may be correlated with environmental conditions (Bastrop et al. 1998; Folino-Rorem et al. 2009; Mackie et al. 2012). In an example of adaptation to a biotic agent, genetic studies strongly suggest that the parasitic castrating barnacle *Loxothylacus panopaei* is in fact a species complex with lineage-specific infection of its mud crab hosts (Kruse and Hare 2007; Kruse et al. 2011). Comparisons of congeneric native and invasive species across several taxa have shown that the invaders often demonstrate higher tolerance of stresses than their native counterparts (Lenz et al. 2011; Sorte et al. 2011). The most comprehensive set of studies on adaptive differences between invasive and native congeners comes from the blue mussels *Mytilus trossulus* and *M. galloprovincialis*, which will be discussed at length in the following section (see Table 2 for references).

Quantitative evidence for intraspecific genetic adaptation in marine invasion has focused on response to two broad environmental stresses: temperature and pollution (Table 1). With an accelerating interest in the impacts of climate change, temperature has become an increasingly well-studied trait, both

Table 2 Studies suggesting adaptation between congeneric species

Genus	PLD	Comparison	Factor	Trait or fitness proxy	Reference
<i>Marenzelleria</i>	4 weeks	I	Salinity	Genetic lineage	Bastrop et al. (1998)
<i>Mytilus</i>	2–5 weeks	NI	Temperature	Survival, growth, behavior, heart rate, enzyme kinetics, gene sequence and function, gene expression, DNA damage, membrane stability, protein activation	Hofmann and Somero (1996), Braby and Somero (2006), Fields et al. (2006), Schneider (2008), Lockwood et al. (2010), Lockwood and Somero (2011, 2012), Yao and Somero (2012), Dowd and Somero (2013)
<i>Mytilus</i>	2–5 weeks	NI	Salinity	Heart rate, survival	Braby and Somero (2006)
<i>Loxothylacus</i> ^a	2 days	NI	Host species	Genetic lineage	Kruse and Hare (2007), Kruse et al. (2011)
<i>Bugula</i>	1 day	I	Depth	Chemotype, symbiont type, genetic lineage	Davidson and Haygood (1999)
<i>Watersipora</i>	1 day	I	Temperature	Genetic lineage	Mackie et al. (2012)
<i>Cordylophora</i>	1 day	I	Salinity	Genetic lineage	Folino-Rorem et al. (2009)
<i>Gammarus</i>	None	NI	Temperature	Survival	Lenz et al. (2011)
<i>Littorina</i>	None	NI	Temperature	Responsiveness	Sorte et al. (2011)

PLD pelagic larval duration, *Comparison* whether the study compared multiple invasive lineages (I) or invasive and non-invasive sister species (NI)

^a Currently considered a single species, *L. panopaei*, but very likely to comprise a cryptic species complex

generally and within the invasion literature. A recent study of four fouling species (*Bugula neritina*, *Botrylloides violaceus*, *Diplosoma listerianum*, and *Botryllus schlosseri*) found differences in temperature tolerance between populations occupying different thermal environments in discrete regions of their invasive range (Sorte et al. 2011), and another study found a cline in survival and growth of *Botrylloides* spp. across a thermal gradient in its invasive range (Grosholz 2001). However, evidence for thermal adaptation is not limited to species, like these, with little to no time spent in a pelagic larval stage. The mud crab *Rhithropanopaeus harrisii* showed thermal differences in larval development between populations (Laughlin and French 1989), and the high-gene flow European green crab *Carcinus maenas* differed in its adult cardiac response to temperature across multiple populations (Tepolt and Somero 2014). There is also evidence for thermal adaptation in the snails *Littorina littorea* and *L. saxatilis* and the oyster *Crassostrea virginica* (Dittman 1997; Dittman et al. 1998; Sokolova and Pörtner 2001; Sorte et al. 2011).

Another area of quantitative study has focused on potential genetic adaptation to pollution, primarily copper compounds (Table 1). Copper is used extensively to coat the hulls of ships in an effort to reduce

fouling, as it is a potent toxin. However, there is a growing body of work suggesting that invasive fouling species may be evolving copper tolerance in response (Piola et al. 2009), which may lend them a competitive advantage over native fouling species (Crooks et al. 2010). Evidence for copper tolerance adaptation in invasive fouling species comes from two bryozoans, *B. neritina* and *Watersipora subtorquata*, and the tunicate *Styela plicata* (Piola and Johnston 2006; Galletly et al. 2007; McKenzie et al. 2011, 2012). Outside of the fouling community, different performance in high-pollution conditions has been recorded for high- and low-pollution populations of the barnacle *Amphibalanus amphitrite*, the clam *Venerupis philippinarum*, and the snail *L. saxatilis* (Daka and Hawkins 2004; Paul-Pont et al. 2010; Romano et al. 2010).

Aside from temperature and pollution, a smaller pool of studies has examined potential adaptation to other selective factors. Salinity tolerance has been studied extensively in the copepod *Eurytemora affinis*, which will be discussed shortly as a case study for adaptive potential (Lee 1999; Lee et al. 2011, 2012). In addition, differences in larval salinity tolerance have been documented between native and invasive population of the mud crab *R. harrisii* (Laughlin and French

1989). Environmental factors primarily associated with different tidal heights have received a great deal of attention in *L. saxatilis*, which in its native range has become something of a model system for ecological divergence (see Table 1 for studies). Different resistance to parasitism has been observed between invasive populations of *V. philippinarum* (Paul-Pont et al. 2010), and the copepod *Acartia tonsa* has shown divergence in response to hypoxia (Decker et al. 2003). The alga *Asparagopsis armata* has shown morphological differences in its native range along gradients of light (Monro and Poore 2009). The dearth of studies outside of temperature and pollution suggests a likely research bias, as these are by no means the only strong selective agents in the sea.

Another class of studies suggesting adaptive differences in invasion is largely genetic (Table 3). In some cases, phylogeographic approaches have been used to link different mitochondrial lineages with environmental differences. For example, the green bagmussel *Musculista senhousia* in its invasive range on the west coast of North America is represented by two distinct mitochondrial lineages that are found in different native thermal environments (Asif and Krug 2012). Similar gene-environment correlation was found in the barnacle *Balanus glandula*, using sequence data from one mitochondrial and one nuclear gene (Geller et al. 2008). In the green crab, *C. maenas*, the center of a mitochondrial cline corresponded with a known north-south biogeographic break in its native European range (Roman and Palumbi 2004; Darling et al. 2008). As with many quantitative studies, these results are suggestive but by no means diagnostic of adaptation. In many cases, these phylogeographic patterns could also be caused by invasion dynamics unrelated to adaptation, such as differences in vector or source populations. In the copepod *E. affinis*, phylogeography was used slightly differently, to demonstrate that recently derived freshwater populations were more closely related to their neighboring native marine populations than to each other, strongly suggesting independent evolution of freshwater tolerance at least five times (Lee 1999).

With sufficient genomic coverage, it is also possible to directly interrogate the genome for signs of selection using outlier analysis. This approach identifies specific genetic markers that are more diverged between populations than is predicted by the background level of divergence over all markers. Because selection acts unevenly on the genome, genetic

positions under selection—and their linked flanking regions—are expected to diverge more rapidly and dramatically than neutral regions of DNA. In marine invasion, outlier analyses have been carried out using microsatellites, AFLPs, and SNPs, scanning tens to thousands of markers (Table 3). An outlier scan in the slipper shell *Crepidula fornicata* examined 327 AFLP and 17 microsatellite markers for loci under selection in a range of native and invasive population comparisons. The authors found eight outlier loci in comparisons across seven native range sites, but no outliers when looking strictly within the invasive range or in comparisons of invasive and native populations together (Riquet et al. 2013). This suggests local adaptation in the species' native range, but shows no evidence for genetic adaptation post-invasion. A scan in the invasive range (plus one native site) of the oyster *Crassostrea gigas* detected five outlier loci out of a total of 278 AFLP, SNP, and microsatellite markers (Rohfritsch et al. 2013). However, given the study design, it is impossible to determine whether selection or demographic history resulted in this pattern. In *L. saxatilis*, a scan of 290 AFLPs showed evidence of selection between populations in the native range at 15 outlier loci (5.2 %); this study did not include any invasive populations (Grahame et al. 2006). Subsequent scans on this species with larger numbers of markers (~2,400 AFLPs and an equal number of SNPs) were consistent with this level of adaptation, finding that 2.2–7.3 % of markers were under selection between tidal heights (Galindo et al. 2009, 2010).

Overall, these outlier studies suggest a low level of genomic differentiation (0–7 % of markers), and little evidence for rapid evolution within invasive ranges. However, the markers used in most of these studies have several limitations that may prevent the detection of selection. First, 200–400 markers may provide insufficient genomic coverage to detect selection, as research in other systems suggests that on average only 5–10 % of the genome may show signs of directional selection (Nosil et al. 2009). This is likely to be lower in many marine systems, with their tendency towards high gene flow (Bradbury et al. 2010; De Wit and Palumbi 2012; Limborg et al. 2012). In addition, because microsatellites and AFLPs are anonymous, they permit only a coarse estimate of overall patterns of selection, although in the case of *L. saxatilis* four promising genetic regions were later sequenced and identified (Wood et al. 2008).

Table 3 Genetic studies suggesting adaptation in marine invasive species

Species	PLD	Marker	Factor	Approach	Range	Conclusions	Reference
<i>Carcinus maenas</i>	4–8 weeks	13 msat; COI	Temperature	Phylogeography	NI	COI lineages correlated with temperature	Roman and Palumbi (2004), Darling et al. (2008)
<i>Musculista senhousia</i>	3 weeks	COI	Temperature	Phylogeography	NI	COI lineages correlated with temperature	Asif and Krug (2012)
<i>Crepidula fornicata</i>	2–7 weeks	327 AFLP; 17 msat	Oceanography	Outlier scan	NI	Potential selection at 2.3 % of markers in native range; no evidence for selection post-invasion	Riquet et al. (2013)
<i>Crassostrea gigas</i>	2–4 weeks	240 AFLP; 30 SNP; 8 msat	Environment	Outlier scan	NI	Potential selection at 1.8 % of markers in invasive range; apparent latitudinal cline at these markers, although this may be an artifact of invasion history	Rohlfritsch et al. (2013)
<i>Balanus glandula</i>	2 weeks	COI; EF1	Temperature	Phylogeography	NI	COI and EF1 lineages correlated with temperature	Geller et al. (2008)
<i>Eurytemora affinis</i>	1–3 weeks	COI	Salinity	Phylogeography	NI	Recent freshwater populations have derived from at least five different mitochondrial lineages	Lee (1999)
<i>Littorina saxatilis</i>	None	290 AFLP (4 seq'd)	Tidal height	Outlier scan Phylogeography	N	Potential selection between high and low tidal height ecomorphs at 5.2 % of markers in native range; targeted sequencing of 4 of these markers showed a strong cline with tidal height	Grahame et al. (2006), Wood et al. (2008)
<i>Littorina saxatilis</i>	None	2356 AFLP; 4 msat	Tidal height	Outlier scan	N	Potential selection between high and low tidal height ecomorphs at 2.2–3.8 % of markers in three different native range populations	Galindo et al. (2009)
<i>Littorina saxatilis</i>	None	2454 SNP	Tidal height	Outlier scan	N	Potential selection between high and low tidal height ecomorphs at 7.3 % of markers in native range	Galindo et al. (2010)

PLD pelagic larval duration, Marker type of genetic marker used (msat microsatellite, COI cytochrome oxidase subunit I sequencing, SNP single nucleotide polymorphism, AFLP amplified fragment-length polymorphism, EF1 elongation factor 1 α sequencing), Range whether the study was carried out in the species' native (N) or invasive (I) range or both (NI)

However, one recent study on *L. saxatilis* employed a panel of 2,454 SNPs and was able to detect potential selection at 7 % of markers, many of which could be identified as belonging to specific genes (Galindo et al. 2010). Studies like these suggest that the drawbacks of many commonly-used approaches to identifying selection may be obviated as next-generation sequencing approaches gain broader use within the marine invasion community. Next-generation sequencing permits outlier analysis at orders of magnitude more locations in the genome, using markers whose position in specific genes are often known. These technologies have the potential to change the way we look at genetic adaptation in invasion, and I will discuss them further later in this paper.

Of course, these approaches are most powerful when combined. Using a purely quantitative approach, it is very difficult (though not impossible) to prove that an ecological or physiological difference between populations is genetically based. Using genetics alone may suggest selection between populations, but without ecological or physiological context, it is impossible to identify a causal agent for that selection. Ideally, future studies will examine adaptation in marine invasion using multiple lines of evidence in concert.

Case studies

Adaptation underlying invasion: enzymatic differences in *Mytilus* congeners

Studying invasive and non-invasive sister species, or congeners, can shed light onto the question of what makes a species invasive, potentially aiding in the prediction of future invasions. Further, congeneric species demonstrating a significant trait difference can help determine the magnitude of genetic change required for a trait shift. One comprehensive example is that of the blue mussels *Mytilus galloprovincialis* and *M. trossulus* along the west coast of North America. *M. trossulus* is native to the north Pacific, and originally spanned Baja California, Mexico through Alaska, USA in the eastern North Pacific (Suchanek et al. 1997). In contrast, *M. galloprovincialis* is native to the Mediterranean Sea. At some point in the early to mid-1900s, *M. galloprovincialis* was introduced to southern California and has subsequently replaced *M. trossulus* in the southern extent of

its range through central California, where the two species hybridize extensively (Rawson et al. 1999). Due to the morphological similarity between these congeners, the invasion was only detected in the 1980s, after it was already well-established, using molecular tools (McDonald and Koehn 1988; Geller et al. 1994).

The biogeographic origin and west coast distribution of the *Mytilus* congeners suggested an adaptive difference between the species, with the Mediterranean-derived invasive *M. galloprovincialis* being more warm-adapted and the northern Pacific-derived native *M. trossulus* being more cold-adapted. This scenario was consistent with the invasion of *M. galloprovincialis* into Mexico and southern California without any apparent establishment in points north, despite evidence that *M. galloprovincialis* larvae were regularly arriving in an Oregon estuary (Geller et al. 1994). Physiological studies lent weight to this hypothesis, showing differences between the congeners (e.g. in the heat shock response, gene expression changes in response to temperature and salinity, and cardiac physiology) that were consistent with a history of adaptation to low (*M. trossulus*) or high (*M. galloprovincialis*) temperatures (Hofmann and Somero 1996; Braby and Somero 2006; Lockwood and Somero 2011).

Building on this rich data set, further experiments identified molecular differences between these congeneric species that might influence their biogeographies. For example, enzyme studies demonstrated a role for the metabolic enzyme cytosolic malate dehydrogenase (cMDH) in mediating differences between the sister species. Evidence from enzyme kinetics suggested that cMDH from *M. trossulus* was cold-adapted relative to *M. galloprovincialis*, and direct sequencing of the cMDH gene showed that the two protein orthologs differed by only two amino acids, one of which was not conserved (and thus more likely to be a selective target) (Fields et al. 2006). In a particularly elegant conclusion, the authors of this study used site-directed mutagenesis to create recombinant *M. trossulus* cMDH proteins that carried the *M. galloprovincialis* substitution, and found that this single amino acid change accounted for the biochemical differences observed between the orthologs (Fields et al. 2006). A later study examined five other enzymes with important roles in ATP production, and found that one of these—isocitrate dehydrogenase

(IDH)—also exhibited significantly different enzyme kinetics between species (Lockwood and Somero 2012). Two non-conserved amino acid differences separated the species, and functional modeling predicted that these two residues were sufficient to change the function of IDH's active site.

The *Mytilus* congeners provide an excellent example of a “gold standard” of adaptation research, linking differences in biogeographic distribution, physiology, and genetic sequence. The mutagenesis experiments are especially useful, as they move the relationship between sequence and physiology beyond the correlative to the causal. While these differences in cMDH and IDH may not represent the full extent of interspecific divergence, they have likely contributed to observed differences in thermal physiology. The warm adaptation of *M. galloprovincialis* appears to have facilitated its initial invasion, and suggests that the species is likely to continue its march up the coast as global temperatures increase. If we view local adaptation as a step along a continuum towards speciation, studies of congeneric invasive-native pairs can tell us the potential “end game” of local adaptation. The example of the *Mytilus* species suggests that, in protein systems at least, very little variation is required for a concrete, adaptive shift. Based on current evidence, only a minority of proteins have changed their thermal optima and the number of amino acid substitutions needed to effect adaptive change is as small as one.

Local adaptation: *Carcinus maenas* thermal biology

Moving along the adaptation continuum, we can examine potentially adaptive differences between populations of the same invasive species inhabiting different environments. Highly successful invasive species are frequently able to survive and thrive across substantial environmental gradients (e.g. of temperature or pollution), presumably exposing them to very different selective environments. Currently, most predictive models of range expansion are based on the assumption that all populations perform equally well in the environmental conditions encountered by any populations—that is to say, the premise is that all populations of a species are interchangeable in their invasion ability. Local adaptation challenges that assumption, because locally-adapted populations

may be predisposed to successfully invade only those locations where the environment is a sufficiently good match.

As an example, I present the European green crab (*Carcinus maenas*) in its native range in Europe and its invasive range along the east coast of North America. The east coast population was founded circa 1817 around the Long Island area (Carlton and Cohen 2003), and slowly spread down the coast to the Chesapeake Bay, where it is believed to be limited by biotic interactions with native blue crabs (deRivera et al. 2005). At the same time, the species spread north through the Gulf of Maine to southern Nova Scotia. This remained the northern limit of the species' range until approximately the 1980s, when it swiftly spread up the coast, making the jump to Newfoundland, the current northern extent of its range, circa 2007 (Roman 2006; Blakeslee et al. 2010). Sequencing of the mitochondrial COI gene suggested a break in the native range between northern and southern Europe, centered near the Netherlands (Roman and Palumbi 2004); this break was not detected with microsatellites (Darling et al. 2008). Genetic work also revealed that the rapid northern expansion of the species along the east coast was coincident with a second introduction from the native range. Genetic approaches were able to trace the original east coast invasion to a source in south/central Europe, while the second introduction into the northeast was derived from a distinct source in northern Europe (Roman 2006; Darling et al. 2008).

The genetic break between north and south in the native range, coupled with the rapid expansion of the east coast population after an infusion of new genetic material from northern Europe, has led to speculation that *C. maenas* may be locally adapted to temperature in its native range. In turn, this local adaptation may have dictated the invasive range available to the species along the east coast. The original invasion, with its south/central European source, spread along the warmer parts of the east coast range, but may have been thermally limited by the bitter winters in the Canadian Maritime provinces. The second introduction, in contrast, may have been adapted to the cold in its native northern Europe, and was able to expand into environments that had been unavailable to the initial invasion. An alternative hypothesis posits that the second introduction facilitated range spread not because of any difference in tolerance, but simply because the location of its introduction bypassed a

current-related barrier to northern spread (Pringle et al. 2011). However, recent physiological research supports the local adaptation hypothesis, finding that crabs from Portugal were more heat- and less cold-tolerant than crabs from Norway. This pattern was recapitulated in the east coast range, where crabs from Newfoundland (derived in part from the second invasion) were more cold- and less heat-tolerant than crabs from New Jersey (derived entirely from the initial invasion) (Tepolt and Somero 2014).

Taken together, the physiological, biogeographic, and genetic evidence all suggest that local adaptation may have played a critical role in the establishment and spread of green crabs along the east coast. Ongoing work is using transcriptome sequencing to identify specific genes and pathways that might be under selection between populations, in an attempt to more explicitly link genotype and phenotype in the species (CKT and SR Palumbi, pers. comm.). Overall, the evidence from this species suggests the importance of considering local adaptation when considering invasion risk. Although *C. maenas* was already established along the east coast, ongoing propagule transport from different sources may have facilitated the spread of the species beyond its previous environmental limits.

Adaptive potential: repeated freshwater transitions in *Eurytemora affinis*

At the far end of the adaptive continuum lies adaptive potential: the ability for populations to evolve in response to future selective pressures. In invasion, adaptive potential is likely primarily derived from standing genetic diversity in a population, resulting in heritable fitness differences between individuals and providing the raw material for selection. Adaptive potential is also of great importance in invasion biology, in part because of the modeling limitations described above. In addition to neglecting adaptive population-level differences, most models also assume that a species will remain in its niche and will not shift its environmental preferences or tolerances in a new range. However, there is increasing evidence, particularly in the terrestrial literature, that invasive species may in fact have a surprising capacity for adaptive shifts (Hill et al. 2013; Morey et al. 2013). By neglecting the potential for future adaptation, we may be severely miscalculating the potential for invasion and range expansion in invaders.

In the marine realm, a prime example is the copepod *Eurytemora affinis*. *E. affinis* is an estuarine species that is widespread throughout the northern hemisphere, and has adapted to freshwater environments at least five independent times in the last century (Lee 1999). Many of its documented freshwater invasions are into reservoirs and other man-made bodies of water with known creation dates, allowing an upper estimate of the date of these shifts, which are generally under 100 years (Lee 1999). Osmotic stress is a major barrier to many species, and acts as a strong selective force against salt to freshwater transitions (Lee and Bell 1999). Given its remarkable, repeated success at making this transition, *E. affinis* provides an excellent example of a rapid, likely-evolved adaptive shift in invasion.

Mitochondrial sequencing of many brackish and freshwater populations has been used to determine the evolutionary origin of each freshwater population, assigning each to a likely brackish source (Lee 1999). This information, in turn, was used to identify sets of populations consisting of an ancestral brackish population and its recently-transitioned freshwater daughter population(s). By comparing ancestral and derived populations, particularly for multiple independent transitions, researchers have been able to examine the nature and magnitude of these shifts. Using this approach, several studies have demonstrated significant shifts in survival, hemolymph osmolality, and ion-pumping enzyme activity between sister populations (Lee et al. 2011, 2012). While causal genetic differences have not been explored, the genetic tracing of different freshwater-tolerant populations to multiple ancestral brackish populations suggests that some kind of parallel adaptive mechanism may be at work.

Perhaps the most interesting part of this system, however, is that these shifts have been recapitulated in approximately 12 generations using laboratory selection experiments. In a study measuring ion-motive enzyme activity, two ancestral brackish populations were maintained in the laboratory at decreasing salinities for seven generations, and then held in freshwater conditions for an additional four to five generations (Lee et al. 2011). These laboratory-selected populations were measured for enzyme activity alongside ancestral brackish and derived freshwater populations from the field. Both selected lines showed performance equivalent to the field-derived freshwater populations, with significant shifts

away from the performance of the brackish population from which they were created (Lee et al. 2011). While not explicitly genetic, this experimental approach provides evidence for a true adaptive genetic basis for freshwater tolerance, and suggests that the time frame for such adaptation may be very rapid.

Looking forward: next-generation sequencing

There is increasing evidence for genetic adaptation at all scales in marine invasion, and rapid climate change suggests an urgent need to incorporate adaptation into predictions of future invasion. However, our understanding of adaptation in marine invasions is still incomplete, greatly hampering our ability to incorporate this important process into model prediction. Sophisticated molecular tools have traditionally been the province of well-characterized species or those closely related to model organisms. In some cases, exploring genes and pathways already well-understood in other systems can uncover parallel evolution in an invasion. However, marine invasions have largely been confined to traditional population genetics tools—typically microsatellites, AFLPs, and direct sequencing of select genes (often mitochondrial COI). These markers can yield important insights into invasion pathways and structure, but are severely limited in their ability to make a concrete connection between genes and adaptive traits.

In the past 5 years, emerging next-generation sequencing technologies are opening up the field of adaptive evolution in many non-model systems (Eklom and Galindo 2011). Recent studies on high-dispersal marine fisheries species have shown genetic differentiation at putatively adaptive loci, even when there is high gene flow and little or no divergence at neutral markers (Nielsen et al. 2009a). For example, markers derived from transcriptome sequencing for the Atlantic herring have revealed significant population structure at select loci (Corander et al. 2013; Lamichhaney et al. 2012). In some cases, population-level frequency of these putatively selected markers is correlated with environmental conditions, notably salinity and temperature (Lamichhaney et al. 2012; Limborg et al. 2012). In the genome-enabled purple sea urchin, suites of genes have been identified which correlate with environmental temperature and pH (Pespeni and Palumbi 2013; Pespeni et al. 2013).

Evidence for previously-cryptic structure has also been observed in Atlantic cod (Nielsen et al. 2009b; Bradbury et al. 2010) and Pacific lamprey (Hess et al. 2013). While these approaches have, to date, rarely been applied to marine invasive species, they have tremendous potential to revolutionize the field.

Next-generation sequencing approaches are now widely used in non-model organisms, although the availability of genomic resources restricts the specific techniques that can be easily used for a given species (Stillman et al. 2008; Nielsen et al. 2009a). To date there are published genomes for five invasive marine species: the ctenophore *Mnemiopsis leidyi*; the sea anemone *Nematostella vectensis*; the tunicates *Ciona intestinalis* and *C. savignyi*; and the oyster *Crassostrea gigas*. For these genome-enabled species, many approaches are available, including genome resequencing and targeted sequence capture (Pespeni et al. 2010; Eklom and Galindo 2011).

For the majority of species, for which a closely-related reference genome is not yet available, messenger RNA sequencing (mRNA-Seq) is one of the most widely-used techniques. In this approach, messenger RNA is converted to complementary DNA and sequenced, giving a record of the sequence of genes being transcribed at the time of preservation (Morozova et al. 2009; Wang et al. 2009). The advantages of mRNA-Seq are that it does not require a genome (gene transcripts can be assembled de novo), is fairly simple to perform (De Wit et al. 2012), markers can usually be assigned to known genes, and it can yield thousands to tens of thousands of well-genotyped polymorphic SNPs for downstream analysis (De Wit and Palumbi 2012). In addition, studies can be carried out in such a way as to also provide gene expression information, thus giving a bit more bang for the buck (De Wit et al. 2012). The disadvantages of mRNA-seq are that it is relatively more expensive and requires significantly more computational resources and expertise than traditional approaches, it can be difficult to reliably assemble a paralog-free de novo transcriptome, and since this approach is reliant on mRNA, appropriate experimental design and consistency in sampling are critical (De Wit et al. 2012).

Despite these caveats, sequencing technology is rapidly becoming easier and cheaper, and it seems likely that next-generation approaches will soon surpass traditional approaches. The most useful of these techniques pinpoint specific genes and

pathways, allowing researchers to identify molecular mechanisms that may be subject to adaptation so that they can be targeted for future work. In addition, the large data sets generated by these approaches can begin to disentangle putative selective targets from neutral background divergence (Hess et al. 2013; McEwan et al. 2013). While NGS approaches do not offer definitive and exhaustive evidence of selection in a system, they are nonetheless highly valuable for comparing patterns of divergence and generating hypotheses about the role and targets of selection in non-model systems (Ellegren 2008; Ekblom and Galindo 2011).

Ideally, NGS approaches will be used in concert with physiological, ecological, environmental, and other data to create a more nuanced and integrated picture of genetic adaptation in marine invasion. Currently, the ability to identify genes and pathways is limited by the available databases, which are built on model organism data. Genome sequencing itself is becoming more tractable, and although these resources are currently limited, the increasing number of taxonomically-diverse, good genomes will only improve the utility of next-generation sequencing (Ellegren 2014). Despite these limitations, I suggest that next-generation sequencing holds tremendous promise for the study of genetic adaptation in marine invasion.

Synthesis

Adaptation is a vitally important and dynamic process, and an increasing body of evidence suggests that it may play an important role in marine invasion on a number of levels. Management that explicitly considers evolutionary processes may be beneficial, particularly given the strong selective forces imposed by rapid climate change (Lankau et al. 2011). Under any form of anthropogenic change, species will have to move, adapt, or die. Invasive species are likely to be successful at both moving and adapting, and we should not neglect the potential for rapid adaptation as we attempt to predict the future.

Genetic tools can give us crucial insights into these processes. At the largest scale, molecular tools can identify cryptic species and introductions, as in the *Mytilus* example above. Parsing the boundaries of cryptic species can have important management

implications (Le Roux and Wieczorek 2009; Geller et al. 2010). In some cases, cryptic lineages may have distinct environmental tolerances (Mackie et al. 2012), which are masked when these lineages are lumped together into a single management unit. Understanding the true relationship between a species' tolerance and its spread may help to direct management efforts towards the highest-risk sources and vectors.

Where physiological data are also available for invasive or potentially invasive species, as in the case of *Mytilus*, it can be used to predict the potential spread of these species, particularly under climate change (Lockwood and Somero 2011). Broadly speaking, we might predict that invasive species pre-adapted to high or highly variable temperatures in their native ranges may be poised for successful invasion in the future. In this case, advantageous adaptation may be more a function of biogeography than any specific invasion-promoting evolution (e.g. Stachowicz et al. 2002). In addition to distinguishing between evolutionarily distinct (but morphologically cryptic) lineages, molecular tools can help identify the magnitude and nature of the genetic differences contributing to differences in environmental tolerances (e.g. *Mytilus* enzyme kinetics).

Within a species, molecular techniques are increasingly highlighting the presence of local adaptation in the oceans, even in species with high dispersal. While quantitative studies have strongly suggested local adaptation to a number of factors, in most cases these results cannot be disentangled from non-heritable effects such as plasticity and maternal effects. Identifying selection at the sequence level is crucial to proving that populations are truly locally adapted, which means that different populations may have a very different ability to successfully invade a new range. This difference in invasion risk suggests that current models may under-perform because they are built on the assumption that all populations are interchangeable.

Like local adaptation, adaptive potential is seldom considered in predictions of invasion spread (Whitney and Gabler 2008). However, several prominent examples of rapid adaptation have come from the terrestrial invasion literature (Phillips et al. 2006; Balanyá et al. 2009), and there is evidence that marine species may also have the potential to evolve quickly in response to novel environments (McKenzie et al. 2011). Such case studies in rapid adaptation are likely based largely on

standing genetic variation rather than new mutations, as was the case with repeated marine to freshwater transitions in the extensively-studied stickleback system (Colosimo et al. 2005; Jones et al. 2012). Standing variation is believed to be particularly important when genetic diversity is relatively high and selective pressures are not novel (reviewed in Hendry 2013). While bottlenecks may reduce diversity in invasive populations, multiple introductions—as demonstrated in several prominent invasions—can increase diversity and produce populations with standing genetic variation that reflects a broader range of past selective environments than was present in any single native population (reviewed in Roman and Darling 2007).

By looking at the molecular level, we can directly interrogate adaptation, and get a deeper sense of the genetic mechanisms underlying these questions. While genetic adaptation is rarely factored into marine invasion policy, molecular tools have played a greater role in understanding and managing terrestrial plant and agricultural pest invasions (Thrall et al. 2011; Kirk et al. 2013). For example, a genomic study of the invasive fruit-crop pest *Drosophila suzukii* has led to insights on the pre-adaptations that have made the species a particularly successful invader, and identified promising genetic targets for control efforts (Ometto et al. 2013). Going one step further, knowledge of the specific genes and mutations underlying resistance to the *Bacillus thuringiensis* (Bt) transgene in agricultural crops has been a vital component of invasive pest management strategies (Carrière et al. 2010; Downes and Mahon 2012; Gassmann 2012).

An increasing understanding of adaptation in invasion, built on a genetic framework, holds promise for greatly improving predictions of invasion risk (Kearney and Porter 2009). This is especially important as climate changes, potentially facilitating invasions by species that are pre-adapted or able to adapt quickly to new or fluctuating conditions (Sorte et al. 2013). Genetic approaches, particularly when used in concert with physiological and ecological tools, can shed light on the mechanisms and dynamics of these adaptations. Ultimately, understanding the role of genetic adaptation in the establishment and spread of marine invasions may allow the development of more effective management strategies and better allocation of resources (Lodge et al. 2006).

Acknowledgments I wish to thank Drs. Steve Palumbi and George Somero and two anonymous reviewers for their insightful comments on previous versions of this manuscript, and Drs. Alison Haupt and John Darling for their helpful overview of some of the tables. I was supported by a fellowship from the Stanford Center for Computational, Evolutionary, and Human Genomics; I greatly appreciate their support. The publication of this paper is supported by CONISMA, the Italian National Interuniversity Consortium for Marine Sciences, which received funding from the European Community's Seventh Framework Programme (FP7/2007–2013) for the project VECTORS (<http://www.marine-vectors.eu>). This paper stems from the International workshop MOLTOOLS (Molecular Tools for Monitoring Marine Invasive Species), held in Lecce, Italy, in September 2012.

References

- Asif JH, Krug PJ (2012) Lineage distribution and barriers to gene flow among populations of the globally invasive marine mussel *Musculista senhousia*. *Biol Invasions* 14:1431–1444
- Atkins KE, Travis MJM (2010) Local adaptation and the evolution of species' ranges under climate change. *J Theor Biol* 266:449–457
- Balanyá J, Oller JM, Huey RB, Gilchrist GW, Serra L (2006) Global genetic change tracks global climate warming in *Drosophila subobscura*. *Science* 313:1773–1775
- Balanyá J, Huey RB, Gilchrist GW, Serra L (2009) The chromosomal polymorphism of *Drosophila subobscura*: a microevolutionary weapon to monitor global change. *Heredity* 103:364–367
- Bastrop R, Jürss K, Sturmbauer C (1998) Cryptic species in a marine polychaete and their independent introduction from North America to Europe. *Mol Biol Evol* 15:97–103
- Blackburn TM (2008) Using aliens to explore how our planet works. *Proc Natl Acad Sci USA* 105:9–10
- Blakeslee AMH, McKenzie CH, Darling JA, Byers JE, Pringle JM, Roman J (2010) A hitchhiker's guide to the Maritimes: anthropogenic transport facilitates long-distance dispersal of an invasive marine crab to Newfoundland. *Divers Distrib* 16:879–891
- Braby CE, Somero GN (2006) Following the heart: temperature and salinity effects on heart rate in native and invasive species of blue mussels (genus *Mytilus*). *J Exp Biol* 209:2554–2566
- Bradbury IR, Hubert S, Higgins B et al (2010) Parallel adaptive evolution of Atlantic cod on both sides of the Atlantic Ocean in response to temperature. *Proc R Soc B* 277:3725–3734
- Carlton JT, Cohen AN (2003) Episodic global dispersal in shallow water marine organisms: the case history of the European shore crabs *Carcinus maenas* and *Carcinus aestuarii*. *J Biogeogr* 30:1809–1820
- Carrière Y, Crowder DW, Tabashnik BE (2010) Evolutionary ecology of insect adaptation to Bt crops. *Evol Appl* 3:561–573
- Chown SL, Slabber S, McGeoch MA, Janion C, Leinaas HP (2007) Phenotypic plasticity mediates climate change

- responses among invasive and indigenous arthropods. *Proc R Soc B* 274:2531–2537
- Chown SL, Hoffmann AA, Kristensen TN, Angilletta MJ Jr, Stenseth NC, Pertoldi C (2010) Adapting to climate change: a perspective from evolutionary physiology. *Climate Res* 43:3–15
- Colosimo PF, Hosemann KE, Balabhadra S et al (2005) Widespread parallel evolution in sticklebacks by repeated fixation of Ectodysplasin alleles. *Science* 307:1928–1933
- Compton TJ, Leathwick JR, Inglis GJ (2010) Thermogeography predicts the potential global range of the invasive European green crab (*Carcinus maenas*). *Divers Distrib* 16:243–255
- Conover DO, Clarke LM, Munch SB, Wagner GN (2006) Spatial and temporal scales of adaptive divergence in marine fishes and the implications for conservation. *J Fish Biol* 69:21–47
- Corander J, Majander KK, Cheng L, Merilä J (2013) High degree of cryptic population differentiation in the Baltic Sea herring *Clupea harengus*. *Mol Ecol* 22:2931–2940
- Crooks JA, Chang AL, Ruiz GM (2010) Aquatic pollution increases the relative success of invasive species. *Biol Invasions* 13:165–176
- Daka ER, Hawkins SJ (2004) Tolerance to heavy metals in *Littorina saxatilis* from a metal contaminated estuary in the Isle of Man. *J Mar Biol Assoc UK* 84:393–400
- Darling JA, Bagley MJ, Roman J, Tepolt CK, Geller JB (2008) Genetic patterns across multiple introductions of the globally invasive crab genus *Carcinus*. *Mol Ecol* 17:4992–5007
- Davidson SK, Haygood MG (1999) Identification of sibling species of the bryozoan *Bugula neritina* that produce different anticancer bryostatins and harbor distinct strains of the bacterial symbiont “*Candidatus Endobugula sertula*”. *Biol Bull* 196:273–280
- De Wit P, Palumbi SR (2012) Transcriptome-wide polymorphisms of red abalone (*Haliotis rufescens*) reveal patterns of gene flow and local adaptation. *Mol Ecol* 22:2884–2897
- De Wit P, Pespeni MH, Ladner JT, Barshis DJ, Seneca F, Jaris H, Therkildsen NO, Morikawa M, Palumbi SR (2012) The simple fool’s guide to population genomics via RNA-Seq: an introduction to high-throughput sequencing data analysis. *Mol Ecol Resour* 12:1058–1067
- Decker MB, Breitbart DL, Marcus NH (2003) Geographical differences in behavioral responses to hypoxia: local adaptation to an anthropogenic stressor? *Ecol Appl* 13:1104–1109
- deRivera CE, Ruiz GM, Hines AH, Jivoff P (2005) Biotic resistance to invasion: native predator limits abundance and distribution of an introduced crab. *Ecology* 86:3364–3376
- Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR (2008) Impacts of climate warming on terrestrial ectotherms across latitude. *Proc Natl Acad Sci USA* 105:6668–6672
- Dittman DE (1997) Latitudinal compensation in oyster ciliary activity. *Funct Ecol* 11:573–578
- Dittman DE, Ford SE, Haskin HH (1998) Growth patterns in oysters, *Crassostrea virginica*, from different estuaries. *Mar Biol* 132:461–469
- Dowd WW, Somero GN (2013) Behavior and survival of *Mytilus* congeners following episodes of elevated body temperature in air and seawater. *J Exp Biol* 216:502–514
- Downes S, Mahon R (2012) Evolution, ecology and management of resistance in *Helicoverpa* spp. to Bt cotton in Australia. *J Invertebr Pathol* 110:281–286
- Eklblom R, Galindo J (2011) Applications of next generation sequencing in molecular ecology of non-model organisms. *Heredity* 107:1–15
- Elith J, Leathwick JR (2009) Species distribution models: ecological explanation and prediction across space and time. *Annu Rev Ecol Evol S* 40:677–697
- Ellegren H (2008) Comparative genomics and the study of evolution by natural selection. *Mol Ecol* 17:4586–4596
- Ellegren H (2014) Genome sequencing and population genomics in non-model organisms. *Trends Ecol Evol* 29:51–63
- Fields PA, Rudomin EL, Somero GN (2006) Temperature sensitivities of cytosolic malate dehydrogenases from native and invasive species of marine mussels (genus *Mytilus*): sequence-function linkages and correlations with biogeographic distribution. *J Exp Biol* 209:656–667
- Folino-Rorem NC, Darling JA, D’Ausilio CA (2009) Genetic analysis reveals multiple cryptic invasive species of the hydrozoan genus *Cordylophora*. *Biol Invasions* 11:1869–1882
- Galindo J, Morán P, Rolán-Alvarez E (2009) Comparing geographical genetic differentiation between candidate and noncandidate loci for adaptation strengthens support for parallel ecological divergence in the marine snail *Littorina saxatilis*. *Mol Ecol* 18:919–930
- Galindo J, Grahame JW, Butlin RK (2010) An EST-based genome scan using 454 sequencing in the marine snail *Littorina saxatilis*. *J Evol Biol* 23:2004–2016
- Galletly BC, Blows MW, Marshall DJ (2007) Genetic mechanisms of pollution resistance in a marine invertebrate. *Ecol Appl* 17:2290–2297
- Gassmann AJ (2012) Field-evolved resistance to Bt maize by western corn rootworm: predictions from the laboratory and effects in the field. *J Invertebr Pathol* 110:287–293
- Geller JB, Carlton JT, Powers DA (1994) PCR-based detection of mtDNA haplotypes of native and invading mussels on the northeastern Pacific coast: latitudinal pattern of invasion. *Mar Biol* 119:243–249
- Geller J, Sotka E, Kado R, Palumbi SR, Schwindt E (2008) Sources of invasions of a northeastern Pacific acorn barnacle, *Balanus glandula*, in Japan and Argentina. *Mar Ecol Prog Ser* 358:211–218
- Geller JB, Darling JA, Carlton JT (2010) Genetic perspectives on marine biological invasions. *Annu Rev Mar Sci* 2:367–393
- Grahame JW, Wilding CS, Butlin RK (2006) Adaptation to a steep environmental gradient and an associated barrier to gene exchange in *Littorina saxatilis*. *Evolution* 60:268–278
- Grosholz E (2001) Small spatial-scale differentiation among populations of an introduced colonial invertebrate. *Oecologia* 129:58–64
- Hamann A, Aitken SN (2013) Conservation planning under climate change: accounting for adaptive potential and

- migration capacity in species distribution models. *Divers Distrib* 19:268–280
- Hastings A, Cuddington K, Davies KF et al (2005) The spatial spread of invasions: new developments in theory and evidence. *Ecol Lett* 8:91–101
- Hendry AP (2013) Key questions in the genetics and genomics of eco-evolutionary dynamics. *Heredity* 111:456–466
- Hess JE, Campbell NR, Close DA, Docker MF, Narum SR (2013) Population genomics of Pacific lamprey: adaptive variation in a highly dispersive species. *Mol Ecol* 22:2898–2916
- Hewitt CL, Campbell ML (2007) Mechanisms for the prevention of marine bioinvasions for better biosecurity. *Mar Pollut Bull* 55:395–401
- Hill MP, Chown SL, Hoffmann AA (2013) A predicted niche shift corresponds with increased thermal resistance in an invasive mite, *Halotydeus destructor*. *Global Ecol Biogeogr* 22:942–951
- Hofmann GE, Somero GN (1996) Interspecific variation in thermal denaturation of proteins in the congeneric mussels *Mytilus trossulus* and *M. galloprovincialis*: evidence from the heat-shock response and protein ubiquitination. *Mar Biol* 126:65–75
- Janson K (1982) Genetic and environmental effects on the growth rate of *Littorina saxatilis*. *Mar Biol* 69:73–78
- Janson K (1983) Selection and migration in two distinct phenotypes of *Littorina saxatilis* in Sweden. *Oecologia* 59:58–61
- Jiménez-Valverde A, Peterson AT, Soberón J, Overton JM, Aragón P, Lobo JM (2011) Use of niche models in invasive species risk assessments. *Biol Invasions* 13:2785–2797
- Johannesson B, Johannesson K (1996) Population differences in behaviour and morphology in the snail *Littorina saxatilis*: phenotypic plasticity or genetic differentiation? *J Zool* 240:475–493
- Johannesson K, Rolán-Alvarez E, Erlandsson J (1997) Growth rate differences between upper and lower shore ecotypes of the marine snail *Littorina saxatilis* (Olivier) (Gastropoda). *Biol J Linn Soc* 61:267–279
- Jones FC, Grabherr MG, Chan YF et al (2012) The genomic basis of adaptive evolution in threespine sticklebacks. *Nature* 484:55–61
- Kearney M, Porter W (2009) Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. *Ecol Lett* 12:334–350
- Kirk H, Dorn S, Mazzi D (2013) Molecular genetics and genomics generate new insights into invertebrate pest invasions. *Evol Appl* 6:842–856
- Koehn RK, Newell RIE, Immermann F (1980) Maintenance of an aminopeptidase allele cline by natural selection. *Proc Natl Acad Sci USA* 77:5385–5389
- Kruse I, Hare MP (2007) Genetic diversity and expanding nonindigenous range of the rhizocephalan *Loxothylacus panopaei* parasitizing mud crabs in the western north Atlantic. *J Parasitol* 93:575–582
- Kruse I, Hare MP, Hines AH (2011) Genetic relationships of the marine invasive crab parasite *Loxothylacus panopaei*: an analysis of DNA sequence variation, host specificity, and distributional range. *Biol Invasions* 14:701–715
- Kuo ESL, Sanford E (2009) Geographic variation in the upper thermal limits of an intertidal snail: implications for climate envelope models. *Mar Ecol Prog Ser* 388:137–146
- Lamichhane S, Martinez Barrio A, Rafati N et al (2012) Population-scale sequencing reveals genetic differentiation due to local adaptation in Atlantic herring. *Proc Natl Acad Sci USA* 109:19345–19350
- Lankau R, Jørgensen PS, Harris DJ, Sih A (2011) Incorporating evolutionary principles into environmental management and policy. *Evol Appl* 4:315–325
- Laughlin RJ, French W (1989) Differences in responses to factorial combinations of temperature and salinity by zoeae from two geographically isolated populations of the mud crab *Rhithropanopeus harrisi*. *Mar Biol* 102:387–395
- Le Roux J, Wicczorek A (2009) Molecular systematics and population genetics of biological invasions: towards a better understanding of invasive species management. *Ann Appl Biol* 154:1–17
- Lee CE (1999) Rapid and repeated invasions of fresh water by the copepod *Eurytemora affinis*. *Evolution* 53:1423–1434
- Lee CE, Bell MA (1999) Causes and consequences of recent freshwater invasions by saltwater animals. *Trends Ecol Evol* 14:284–288
- Lee CE, Kiergaard M, Gelembiuk GW, Eads BD, Posavi M (2011) Pumping ions: rapid parallel evolution of ionic regulation following habitat invasions. *Evolution* 65:2229–2244
- Lee CE, Posavi M, Charmantier G (2012) Rapid evolution of body fluid regulation following independent invasions into freshwater habitats. *J Evol Biol* 25:625–633
- Lenz M, da Gama BAP, Gerner NV et al (2011) Non-native marine invertebrates are more tolerant towards environmental stress than taxonomically related native species: results from a globally replicated study. *Environ Res* 111:943–952
- Limborg MT, Helyar SJ, De Bruyn M, Taylor MI, Nielsen EE, Ogden R, Carvalho GR, Bekkevold D (2012) Environmental selection on transcriptome-derived SNPs in a high gene flow marine fish, the Atlantic herring (*Clupea harengus*). *Mol Ecol* 21:3686–3703
- Lockwood BL, Somero GN (2011) Invasive and native blue mussels (genus *Mytilus*) on the California coast: the role of physiology in a biological invasion. *J Exp Mar Biol Ecol* 400:167–174
- Lockwood BL, Somero GN (2012) Functional determinants of temperature adaptation in enzymes of cold- versus warm-adapted mussels (genus *Mytilus*). *Mol Biol Evol* 29:3061–3070
- Lockwood BL, Sanders JG, Somero GN (2010) Transcriptomic responses to heat stress in invasive and native blue mussels (genus *Mytilus*): molecular correlates of invasive success. *J Exp Biol* 213:3548
- Lodge DM, Williams S, MacIsaac HJ et al (2006) Biological invasions: recommendations for US policy and management. *Ecol Appl* 16:2035–2054
- Mackie JA, Darling JA, Geller JB (2012) Ecology of cryptic invasions: latitudinal segregation among *Watersipora* (Bryozoa) species. *Sci Rep* 2:1–10
- McDonald JH, Koehn RK (1988) The mussels *Mytilus galloprovincialis* and *M. trossulus* on the Pacific coast of North America. *Mar Biol* 99:111–118
- McEwan JR, Vamosi JC, Rogers SM (2013) Natural selection and neutral evolution jointly drive population divergence between alpine and lowland ecotypes of the allopolyploid

- plant *Anemone multifida* (Ranunculaceae). PLoS ONE 8:e68889
- McKenzie LA, Brooks R, Johnston EL (2011) Heritable pollution tolerance in a marine invader. *Environ Res* 111:926–932
- McKenzie LA, Johnston EL, Brooks R (2012) Using clones and copper to resolve the genetic architecture of metal tolerance in a marine invader. *Ecol Evol* 2:1319–1329
- Molnar JL, Gamboa RL, Revenga C, Spalding MD (2008) Assessing the global threat of invasive species to marine biodiversity. *Front Ecol Environ* 6:485–492
- Monro K, Poore AGB (2009) The evolvability of growth form in a clonal seaweed. *Evolution* 63:3147–3157
- Morey A, Venette R, Hutchison W (2013) Could natural selection change the geographic range limits of light brown apple moth (Lepidoptera, Tortricidae) in North America? *NeoBiota* 18:151–156
- Morozova O, Hirst M, Marra MA (2009) Applications of new sequencing technologies for transcriptome analysis. *Annu Rev Genomics Hum Genet* 10:135–151
- Nielsen EE, Hemmer-Hansen J, Larsen PF, Bekkevold D (2009a) Population genomics of marine fishes: identifying adaptive variation in space and time. *Mol Ecol* 18:3128–3150
- Nielsen EE, Hemmer-Hansen J, Poulsen NA et al (2009b) Genomic signatures of local directional selection in a high gene flow marine organism; the Atlantic cod (*Gadus morhua*). *BMC Evol Biol* 9:276
- Nosil P, Funk DJ, Ortiz-Barrientos D (2009) Divergent selection and heterogeneous genomic divergence. *Mol Ecol* 18:375–402
- O'Neill GA, Hamann A, Wang T (2008) Accounting for population variation improves estimates of the impact of climate change on species' growth and distribution. *J Appl Ecol* 45:1040–1049
- Ometto L, Cestaro A, Ramasamy S et al (2013) Linking genomics and ecology to investigate the complex evolution of an invasive *Drosophila* pest. *Genome Biol Evol* 5:745–757
- Pardo L, Johnson L (2005) Explaining variation in life-history traits: growth rate, size, and fecundity in a marine snail across an environmental gradient lacking predators. *Mar Ecol Prog Ser* 296:229–239
- Paul-Pont I, de Montaudouin X, Gonzalez P, Soudant P, Baudrimont M (2010) How life history contributes to stress response in the Manila clam *Ruditapes philippinarum*. *Environ Sci Pollut R* 17:987–998
- Pertoldi C, Bach L (2007) Evolutionary aspects of climate-induced changes and the need for multidisciplinary. *J Therm Biol* 32:118–124
- Pespeni MH, Palumbi SR (2013) Signals of selection in outlier loci in a widely dispersing species across an environmental mosaic. *Mol Ecol* 22:3580–3597
- Pespeni MH, Oliver TA, Manier MK, Palumbi SR (2010) Restriction Site Tiling Analysis: accurate discovery and quantitative genotyping of genome-wide polymorphisms using nucleotide arrays. *Genome Biol* 11:R44
- Pespeni MH, Chan F, Menge BE, Palumbi SR (2013) Signs of adaptation to local pH conditions across an environmental mosaic in the California Current ecosystem. *Integr Comp Biol* 53:857–870
- Phillips BL, Brown GP, Webb JK, Shine R (2006) Invasion and the evolution of speed in toads. *Nature* 439:803
- Piola RF, Johnston EL (2006) Differential tolerance to metals among populations of the introduced bryozoan *Bugula neritina*. *Mar Biol* 148:997–1010
- Piola RF, Dafforn KA, Johnston EL (2009) The influence of antifouling practices on marine invasions. *Biofouling* 25:633–644
- Preisser EL, Elkinton JS, Abell K (2008) Evolution of increased cold tolerance during range expansion of the elongate hemlock scale *Fiorinia externa* Ferris (Hemiptera: Diaspididae). *Ecol Entomol* 33:709–715
- Pringle JM, Blakeslee AMH, Byers JE, Roman J (2011) Asymmetric dispersal allows an upstream region to control population structure throughout a species' range. *Proc Natl Acad Sci USA* 108:15288–15293
- Rawson PD, Agrawal V, Hilbish TJ (1999) Hybridization between the blue mussels *Mytilus galloprovincialis* and *M. trossulus* along the Pacific coast of North America: evidence for limited introgression. *Mar Biol* 134:201–212
- Riquet F, Daguin-Thiébaud C, Ballenghien M, Bierne N, Viard F (2013) Contrasting patterns of genome-wide polymorphism in the native and invasive range of the marine mollusc *Crepidula fornicata*. *Mol Ecol* 22:1003–1018
- Rohfritsch A, Bierne N, Boudry P, Heurtebise S, Cornette F, Lapégue S (2013) Population genomics shed light on the demographic and adaptive histories of European invasion in the Pacific oyster, *Crassostrea gigas*. *Evol Appl* 6:1064–1078
- Rolán-Alvarez E, Johannesson K, Erlandsson J (1997) The maintenance of a cline in the marine snail *Littorina saxatilis*: the role of home site advantage and hybrid fitness. *Evolution* 51:1838–1847
- Roman J (2006) Diluting the founder effect: cryptic invasions expand a marine invader's range. *Proc R Soc B* 273:2453–2459
- Roman J, Darling JA (2007) Paradox lost: genetic diversity and the success of aquatic invasions. *Trends Ecol Evol* 22:454–464
- Roman J, Palumbi SR (2004) A global invader at home: population structure of the green crab, *Carcinus maenas*, in Europe. *Mol Ecol* 13:2891–2898
- Romano JA, Rittschof D, McClellan-Green PD, Holm ER (2010) Variation in toxicity of copper pyriithione among populations and families of the barnacle, *Balanus amphitrite*. *Biofouling* 26:341–347
- Sanford E, Kelly MW (2011) Local adaptation in marine invertebrates. *Annu Rev Mar Sci* 3:509–535
- Schneider KR (2008) Heat stress in the intertidal: comparing survival and growth of an invasive and native mussel under a variety of thermal conditions. *Biol Bull* 215:253–264
- Sgrò CM, Overgaard J, Kristensen TN, Mitchell KA, Cockerell FE, Hoffmann AA (2010) A comprehensive assessment of geographic variation in heat tolerance and hardening capacity in populations of *Drosophila melanogaster* from eastern Australia. *J Evol Biol* 23:2484–2493
- Sokolova IM, Pörtner HO (2001) Temperature effects on key metabolic enzymes in *Littorina saxatilis* and *L. obtusata* from different latitudes and shore levels. *Mar Biol* 139:113–126

- Sorte CJB, Williams SL, Carlton JT (2010) Marine range shifts and species introductions: comparative spread rates and community impacts. *Global Ecol Biogeogr* 19:303–316
- Sorte CJB, Jones SJ, Miller LP (2011) Geographic variation in temperature tolerance as an indicator of potential population responses to climate change. *J Exp Mar Biol Ecol* 400:209–217
- Sorte CJB, Ibáñez I, Blumenthal DM et al (2013) Poised to prosper? A cross-system comparison of climate change effects on native and non-native species performance. *Ecol Lett* 16:261–270
- Stachowicz JJ, Terwin JR, Whitlatch RB, Osman RW (2002) Linking climate change and biological invasions: ocean warming facilitates nonindigenous species invasions. *Proc Natl Acad Sci USA* 99:15497–15500
- Stillman JH, Colbourne JK, Lee CE et al (2008) Recent advances in crustacean genomics. *Integr Comp Biol* 48:852–868
- Suchanek TH, Geller JB, Kreiser BR, Mitton JB (1997) Zoogeographic distributions of the sibling species *Mytilus galloprovincialis* and *M. trossulus* (Bivalvia: Mytilidae) and their hybrids in the north Pacific. *Biol Bull* 193:187–194
- Tepolt CK, Somero GN (2014) Master of all trades: thermal acclimation and adaptation of cardiac function in a broadly-distributed marine invasive species, the European green crab, *Carcinus maenas*. *J Exp Biol* 217:1129–1138
- Thrall PH, Oakeshott JG, Fitt G et al (2011) Evolution in agriculture: the application of evolutionary approaches to the management of biotic interactions in agro-ecosystems. *Evol Appl* 4:200–215
- Van Doorslaer W, Vanoverbeke J, Duvivier C et al (2009) Local adaptation to higher temperatures reduces immigration success of genotypes from a warmer region in the water flea *Daphnia*. *Global Change Biol* 15:3046–3055
- Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet* 10:57–63
- West-Eberhard MJ (2003) Developmental plasticity and evolution. Oxford University Press, Oxford
- Whitney KD, Gabler CA (2008) Rapid evolution in introduced species, “invasive traits” and recipient communities: challenges for predicting invasive potential. *Divers Distrib* 14:569–580
- Wood HM, Grahame JW, Humphray S, Rogers J, Butlin RK (2008) Sequence differentiation in regions identified by a genome scan for local adaptation. *Mol Ecol* 17:3123–3135
- Yao C-L, Somero GN (2012) The impact of acute temperature stress on hemocytes of invasive and native mussels (*Mytilus galloprovincialis* and *Mytilus californianus*): DNA damage, membrane integrity, apoptosis and signaling pathways. *J Exp Biol* 215:4267–4277