

# What are we missing about marine invasions? Filling in the gaps with evolutionary genomics

C. D. H. Sherman<sup>1</sup> · K. E. Lotterhos<sup>2</sup> · M. F. Richardson<sup>1</sup> · C. K. Tepolt<sup>3</sup> ·  
L. A. Rollins<sup>1</sup> · S. R. Palumbi<sup>4</sup> · A. D. Miller<sup>1,5</sup>

Received: 18 April 2016 / Accepted: 4 August 2016  
© Springer-Verlag Berlin Heidelberg 2016

**Abstract** Research on invasion biology has been largely dominated by studies on the ecological effects of invasion events, although recently, evolutionary processes have been shown to be important to invasion success. This is largely attributed to novel genomic tools that provide new opportunities to unravel the natural history, taxonomy, and invasion pathways of invasive species, as well as the genetic basis of adaptive traits that allow them to expand within and beyond their native range. Despite these advances and the growing literature of genomic research on terrestrial pests, these tools have not been widely applied to marine invasive species. This is in part due to the perception that high levels of dispersal and connectivity in many invasive marine species

can limit the opportunity for local adaptation. However, there is growing evidence that even in species with high dispersal potential, significant site-specific adaptation can occur. We review how these “omic” tools provide unprecedented opportunities to characterise the role of adaptive variation, physiological tolerance, and epigenetic processes in determining the success of marine invaders. Yet, rapid range expansion in invasions can confound the analysis of genomic data, so we also review how data should be properly analysed and carefully interpreted under such circumstances. Although there are a limited number of studies pioneering this research in marine systems, this review highlights how future studies can be designed to integrate ecological and evolutionary information. Such datasets will be imperative for the effective management of marine pests.

---

Responsible Editor: E. Briski.

---

Reviewed by Undisclosed experts.

---

This article is part of the Topical Collection on Invasive Species.

---

✉ C. D. H. Sherman  
craig.sherman@deakin.edu.au

<sup>1</sup> School of Life and Environmental Sciences, Centre for Integrative Ecology, Deakin University, Waurn Ponds Campus, 75 Pigdons Road. Locked Bag 20000, Geelong, VIC 3220, Australia

<sup>2</sup> Department of Marine and Environmental Science, Northeastern University Marine Science Center, 430 Nahant Road, Nahant, MA 01908, USA

<sup>3</sup> Smithsonian Environmental Research Center, 647 Contees Wharf Road, Edgewater, MD 21037, USA

<sup>4</sup> Hopkins Marine Station, Stanford University, Pacific Grove, CA 93950, USA

<sup>5</sup> Pest and Environmental Adaptation Research Group, School of BioSciences, The University of Melbourne, Parkville, VIC 3010, Australia

## Introduction

Marine biological invasions are occurring on an international scale at unprecedented rates, driven by rapid globalisation and increasing maritime trade (Bax et al. 2003; Molnar et al. 2008). These invasions present a significant threat to ecosystem function as introduced species are considered to be a major driver of marine biodiversity loss and ecosystem functioning through the modification of marine habitat, community structure, and dynamics (Grosholz 2002; Bax et al. 2003; Molnar et al. 2008). Marine pests can cause significant economic burdens impacting marine infrastructure, commercial and recreational fisheries, and aquaculture operations, as well as human health (Shiganova 1998; Grosholz 2002; Bax et al. 2003; Hewitt 2003; Lewis et al. 2003; Davidson et al. 2015; Ojaveer et al. 2015). In the USA alone, more than 500 marine introduced species have

been recorded, with an estimated economic impact exceeding \$120 billion annually (Fofonoff et al. 2003; Pimentel et al. 2005). However, it is likely that these figures are grossly underestimated, as only a small fraction of invasive species and their associated impacts are likely to have been recorded (Ruiz et al. 1997). Consequently, preventing and managing the introduction of invasive species has become a major conservation priority with major investments in invasive species and biosecurity research in recent years (Rilov and Crooks 2009).

Genetic tools have long played an important role in invasive species research and are routinely used to confirm species taxonomies, introduction sources, invasion routes, patterns of population connectivity, and demographic history (i.e. populations bottlenecks and expansions) (Booth et al. 2007; Tepolt et al. 2009; Bock et al. 2015; Ab Rahim et al. 2016). Such studies assist greatly in preventing further introduction events, determining the risk of species establishment and expansion, and identifying habitats most at risk of future invasion (Roman and Darling 2007; Dlugosch and Parker 2008; Bock et al. 2015; Dlugosch et al. 2015). However, until now invasive species genetic research has relied largely on the use of a small number of markers that analyse patterns of neutral genetic variation. Neutral genetic variation involves parts of the genome that are not under selection and are particularly useful for gaining insights into species taxonomies, reproductive modes, dispersal capacities, and demographic processes such as patterns of population connectivity (i.e. gene flow), effective population size, and evolutionary history (i.e. historical population bottleneck or expansion events) (Frankham et al. 2009; Hedrick 2011). However, these neutral gene variants do not have any direct effects on population fitness and therefore provide limited information about the adaptive potential of invasive populations (Holderegger et al. 2006; Lal et al. 2016). Adaptive variation reflects the genetic basis of traits that are adapted to the local environment and has traditionally been assessed using common garden and reciprocal transplant experiments (Ayre 1995; Sanford et al. 2003; Hollander et al. 2006; Hutchings et al. 2007; Sherman and Ayre 2008; Landy and Travis 2015). Understanding how invasive species respond and adapt to novel environments is important for predicting the success of establishment and range expansion and thus is crucial for the development of effective management and conservation strategies (Hastings et al. 2005; Hewitt and Campbell 2007; Colautti and Lau 2015).

In contrast to terrestrial taxa, marine species have been considered unable to develop strong local adaptation because of high levels of gene flow between populations that inhabit contrasting environments (Williams 1975; Johnson and Black 1984; Sherman and Ayre 2008; Sanford and Kelly 2011; Colautti and Lau 2015). However, there is

growing evidence that many marine taxa, including invasive species, show evidence of adaptation to local conditions (Sanford and Kelly 2011; Colautti and Lau 2015; Dlugosch et al. 2015; Tepolt 2015). Most of the evidence for genetic adaptation in marine invasive species comes from quantitative studies that have shown divergence in a range of morphological or physiological traits, rather than identifying the genetic loci underlying adaptive traits (reviewed in Tepolt 2015). However, modern DNA sequencing technologies provide an unprecedented opportunity for detecting fine-scale patterns of neutral genetic differentiation and characterising adaptive variation in invasive marine species (Benestan et al. 2015; Blanco-Bercial and Bucklin 2016)—even when there is limited information on either molecular or quantitative trait variation (Hess et al. 2013; Milano et al. 2014). Specifically, this technology enables candidate genes or genomic regions that underlie adaptive differences between populations to be determined and measured directly, rather than relying on indirect measurements associated with more traditional approaches.

In the context of marine invasive species, here we review how genomic (DNA), transcriptomic (RNA), and epigenetic (e.g. cytosine methylation, microRNA) tools allow for the characterisation of molecular variation across large numbers of coding and non-coding genetic markers (hundreds to hundreds of thousands) at the individual, population, and species level (Rius et al. 2015). These approaches are being routinely applied to non-model species, although to date their application to marine invasive species remains limited (Table 1). While the evolutionary genetics of invasive introductions have been extensively covered in recent reviews (Grosholz 2002; Lee 2002; Booth et al. 2007; Sax et al. 2007; Bock et al. 2015; Dlugosch et al. 2015; Rius et al. 2015; Tepolt 2015), these reviews have failed to highlight the issues associated with the statistical analysis of genomic data from invasive species. We review how rapid range expansion in invasions can create characteristic signatures in the genome that may resemble selection, and discuss how data should be properly analysed and carefully interpreted under such circumstances. We conclude that modern sequencing technologies provide for better understanding of the marine invasion processes, and highlight the growing importance of integrating ecological and evolutionary information for effective management of marine pests.

### Estimating patterns of neutral genetic structure in marine invasive species

Understanding patterns of neutral genetic structure can provide valuable insights into species invasive potential. Specifically, estimates of the degree of gene flow and genetic

structuring within and between populations across native and invasive ranges can be used to infer species dispersal capacities, demographic histories, and invasion pathways. Furthermore, gene flow can play a critical role in the environmental adaptation process, and valuable insights can be gained when gene flow patterns are characterised across environmental gradients. For example, gene flow among populations from similar environments can enhance rates of local adaptation to stressful conditions by introducing new genetic variants available for selection (Holt and Gomulkiewicz 1997; Sexton et al. 2014). Conversely, high rates of gene flow between populations from different environments can retard adaptation through the introduction of maladaptive variants (Frankham et al. 2011).

Despite the opportunities that traditional genetic and modern genomic approaches offer for estimating neutral genetic structure, the bulk of the literature in this space is dominated by research on invasive species from terrestrial systems, particularly species associated with agriculture, horticulture, and livestock industries (Kirk et al. 2013; Miller et al. 2013; Hill et al. 2016; Poelchau et al. 2016). Comparatively, investments in marine pest research have been modest in number (Grosholz 2002; Darling et al. 2008; Reusch et al. 2010; Zhan et al. 2015) and likely due to several factors including the logistics of sampling in the marine environment, historical economic drivers, and the difficulty of obtaining reliable neutral population genetic estimates from pest species in both their native and invasive ranges. Estimating neutral genetic structure and population connectivity in marine species is often challenging and precluded by several factors. Marine species are typically characterised by large population sizes, and the degree of connectivity between them is often high compared with species from freshwater and terrestrial systems (Waples 1998; Allendorf et al. 2010). These factors tend to drive high levels of genetic variation and suppress drift processes and rates of genetic differentiation (Whitlock and McCauley 1999a; Hauser and Carvalho 2008).

Because of these demographic characteristics, it is often assumed that insufficient time has passed for many marine species to reach migration–drift equilibrium following the colonisation of post-glacial and invasive range habitats (Hewitt 2004; Herborg et al. 2007; Hauser and Carvalho 2008). Estimates of contemporary population connectivity based on genetic markers might then be inaccurate with the potential to mislead management. For example, recent genetic research on the Chinese mitten crab, *Eriocheir sinensis*, across its invasive European range found evidence of low genetic structuring despite high levels of genetic structuring in its native range. Multiple lines of evidence suggest that these populations have not reached migration–drift equilibrium and that estimates of genetic structure and population connectivity are likely to be inaccurate,

particularly estimates based on traditional measures of genetic differentiation that assume equilibrium (e.g.  $F_{ST}$ ) (Waples 1998; Whitlock and McCauley 1999b; Herborg et al. 2007). In such cases, it has been suggested that alternative analytical approaches including non-equilibrium Bayesian approaches, direct dispersal tests (i.e. relatedness and spatial autocorrelation analyses and assignment tests), and entropy-based methods can help overcome the limitations of  $F_{ST}$  (Pearse and Crandall 2004; Herborg et al. 2007; Chao et al. 2015). Even with the development of these alternative statistical approaches, our ability to differentiate weak genetic differentiation from statistical artefacts remains a challenge (Waples 1998; Hauser and Carvalho 2008).

Despite these challenges for understand weak genetic structure, the large number of genetic markers ascertained by NGS approaches for populations genetic studies can nevertheless improve inference of population structure. In particular, reduced genome representation sequencing protocols [e.g. restriction site-associated DNA tag sequencing (RAD-Seq) and genotype by sequencing (GBS)] now facilitate genotyping of individuals across populations for hundreds to hundreds of thousands of anonymous genetic markers compared with tens of markers typically afforded by traditional methods (Elshire et al. 2011; Peterson et al. 2012). While the utility of NGS approaches for marine invasive species research has been limited to date (Table 1), the superior performance of high-density NGS-derived markers has been demonstrated on several occasions using native species models. For example, in a recent population genomic analysis of the American lobster (*Homarus americanus*) by Benestan et al. (2015), 10,000 SNP markers were used to detect significant patterns of regional and local genetic structure, despite previous reports of weak genetic differentiation based on traditional and less sensitive markers (e.g. allozymes, RAPDs, and microsatellites). Similarly, a large panel of NGS-derived SNP markers (approximately 12,000) were recently used to describe patterns of population subdivision in the lined seahorse (*Hippocampus erectus*) from the west coast of the USA, where traditional markers failed to do so in previous studies (Boehm et al. 2015). Additionally, Blanco-Bercial and Bucklin (2016) demonstrated the power of high-density RAD-Seq markers combined with non-equilibrium analytical approaches for detecting signals of neutral genetic structure among North Atlantic planktonic copepod (*Centropages typicus*), a species which is characterised by extremely large population sizes and is unlikely to be at a demographic equilibrium. Although NGS approaches have the potential to generate a higher resolution of spatial patterns of genetic structure in marine species, these have only been applied to a handful of marine invasive species to date (Table 1). Consequently, investments in marine pest population genomic research

**Table 1** A selection of studies marine invasive species that highlight the use genetic information for informing invasion biology

Species	Status (native and known invasive ranges)	Genetic/epigenetic information					Context (Invasive, Non-invasive, Both)	References	
		Genome/transcriptome resources	Taxonomic identification	Source/s identification (Single or multiple introductions)	Population structure (Invasive, Native, Both)	Adaptive variation			Gene expression
<i>Asterias amurensis</i>	Japan, Korea, Russia <i>Australia</i>	De novo Transcriptome Assembly	Yes	Single	Both	–	Yes	–	Ward and Andrew (1995), Matsuoka and Hatanaka (1998), Murphy and Evans (1998), Knott and Wray (2000), Yamashita et al. (2005), Richardson and Sherman (2015), Richardson et al. (in press)
<i>Caulerpa taxifolia</i>	Indian Ocean <i>Australia, USA, Mediterranean Sea</i>	De novo Transcriptome Assembly	Yes	Multiple	–	–	Yes	–	Olsen et al. (1998), Benzie et al. (2000), Wiedenmann et al. (2001), Meusnier et al. (2002), Arnaud-Haond et al. (2013), Ranjan et al. (2015)
<i>Carcinus maenas</i>	Europe, Atlantic northwest <i>Africa, North American east coast, North American west coast, South Africa, Japan, Australia, Argentina</i>	De novo Transcriptome Assembly	Yes	Multiple	Both	Yes	Yes	–	Geller et al. (1997), Roman and Palumbi (2004), Roman (2006), Darling et al. (2008), Tepolt et al. (2009), Blakeslee et al. (2010), Kelley et al. (2011), Pringle et al. (2011), Tepolt and Somero (2013), Burden et al. (2014), Darling et al. (2014), Tepolt and Palumbi (2015), Verbruggen et al. (2015)
<i>Eriocheris sinensis</i>	East Asia <i>Europe, US</i>	Genome	Yes	Multiple	Both	–	Yes	–	Herborg et al. (2007), Sui et al. (2009), Hänfling et al. (2011), Li et al. (2013b), Cho et al. (2014), Li et al. (2014), Song et al. (2016)

Table 1 continued

Species	Status (native and known invasive ranges)	Genetic/epigenetic information					Population structure (Invasive, Native, Both)	Adaptive variation	Gene expression	Epigenetic variation	Context (Invasive, Non-invasive, Both)	References
		Genome/transcriptome resources	Taxonomic identification	Source/s identification or multiple introductions	Genome/transcriptome resources	Genome/transcriptome resources						
<i>Mytilus galloprovincialis</i>	Northern and Southern hemisphere	Genome	Yes	Multiple	Both	–	–	Yes	–	Both	McDonald and Koehn (1988), McDonald et al. (1991), Suchanek et al. (1997), Toro (1998), Hilbish et al. (2000), Borsa et al. (2007), Gerard et al. (2008), Lockwood et al. (2010), Westfall and Gardner (2010), Lockwood and Somero (2011), Moreira et al. (2015), Ab Rahim et al. (2016), Mur-garella et al. (2016)	
	<i>North America, South America, South Africa, Australia, New Zealand, Hong Kong, Japan, Korean peninsula</i>	De novo Transcriptome Assembly										
<i>Mnemiopsis leidyi</i>	Atlantic Ocean Black, North, Azov, Caspian and Baltic seas	Genome	Yes	Multiple	Both	Yes	Yes	Yes	Yes	Both	Pang and Martindale (2008), Gorokhova et al. (2009), Reusch et al. (2010), Ghabooli et al. (2011), Schmitzler et al. (2012), Bolte et al. (2013), Ryan et al. (2013), Möller et al. (2014), Bayha et al. (2015), Dabe et al. (2015)	
<i>Undaria pinnatifida</i>	Northeast Asia and Russia <i>Europe, North America, South America and Australasia</i>	De novo Transcriptome Assembly	Yes	Multiple	Both	–	–	Yes	–	Both	Muraoka and Saitoh (2005), Uwai et al. (2005), Voisin et al. (2005), Uwai et al. (2006a), Uwai et al. (2006b), Uwai et al. (2009), Grulois et al. (2011), Li et al. (2013a), Shan et al. (2015a), Shan et al. (2015b), Zhang et al. (2015)	

is expected to greatly assist management by providing a clearer understanding of contemporary patterns of gene flow, genetic structure, and dispersal capacities in both native and invasive ranges.

### Application of genomic tools for measures of adaptive genetic variation

A central goal in invasion genetics is to understand the physiological mechanisms and the source of genetic variation that underpins invasiveness. Some successful invaders arrive well suited to their new environments, others adapt rapidly to local conditions, and yet others experience a long period of quiescence before a population boom (Bock et al. 2015). To elucidate the relative roles of standing genetic variation, new mutation, and introgression in determining the success of invasive lineages, we must first identify the genetic variants that contribute to ecologically important phenotypes. For the first time, NGS technologies now provide the means by which adaptive genetic variation can be estimated in the absence of any prior information on either molecular or quantitative trait variation, although the ideal experiment would combine both top-down and bottom-up genetic approaches. NGS technology enables candidate genes, or closely linked loci, that underlie adaptive differences between populations to be identified, rather than relying on indirect measurements associated with more traditional morphological approaches. Adaptive differentiation can be determined specifically through genome scans for loci that are either directly located in adaptive genes or linked to genes and genomic regions under selection.

To our knowledge, there are no examples of NGS technologies being applied to marine pests for adaptation research in the literature, yet there is a rapidly growing body of the literature on native species. Examples from the literature indicate that adaptive genetic differentiation among populations from different habitat types can be detected even in highly dispersive marine species with little neutral genetic structure. For example, recent genomic studies of Atlantic cod (*Gadus morhua* L.) populations have identified directionally selected loci that are strongly correlated with habitat differences in salinity, oxygen, and temperature (Berg et al. 2015). Subsequent mapping of loci to a reference genome indicates that these genes are likely to be within or closely located to genes associated with osmoregulation, and it is proposed that these adaptive genetic differences contribute to observed patterns of reproductive isolation among populations from different habitats. In another study on European hake (*Merluccius merluccius*), adaptive genetic differentiation was uncovered among populations collected from different sea surface temperatures and salinities (Milano et al. 2014). Different SNP variants

were associated with genes linked to cytoskeleton formation and the regulation of cellular energetics, both of which have been shown to be important for temperature adaptation (Milano et al. 2014). Likewise, despite underlying signatures of panmixia in Pacific lamprey populations (*Entosphenus tridentatus*), adaptive variation was found in this species to be related to geography and life history (Hess et al. 2013). Such studies provide valuable insight into drivers of genetic differentiation among populations and provide a basis for determining mechanisms of adaptation (i.e. plasticity vs. genetic). Furthermore, and as pointed out by Berg et al. (2015), these studies provide a starting point for functional genomic analyses and is a particularly powerful approach for characterising historical patterns of selection and future adaptive capacities (Hoffmann and Willi 2008).

### DNA-based genomic approaches for characterising adaptive variation

The evolutionary study of invasive lineages seeks to identify the source(s) of the invasion, understand the role of population bottlenecks and adaptation in the spread of the invasion, and identify the sources of genetic variation that make invading organisms successful (Bock et al. 2015). Answering these questions will typically require genetic information from a large number of individuals across the invasive and native ranges. Although the cost of sequencing has significantly reduced over the last two decades, the ability to sequence the entire genome of every sampled individual is still prohibitively expensive and poses a significant bioinformatics challenge for most non-model organisms. Thus, we must either reduce the amount of genomic data ascertained per individual, or pool DNA from multiple individuals for whole-genome sequencing (Futschik and Schlotterer 2010; Schlotterer et al. 2014). The choices made in ascertainment have implications for the possible statistical methods that can be employed on the data.

The two most common methods for reduced representation of the genome are RAD-Seq/GBS and RNA-sequencing (RNA-Seq). RAD-Seq has the advantage of being cost-effective at an order of magnitude lower cost than RNA-Seq, yet RNA-Seq has the advantage of focusing on coding regions that are more likely to be of functional importance (discussed below). RAD-Seq works by using restriction–ligation methods to shear, amplify, and sequence only a fraction of the genome (reviewed in Davey et al. 2011; Puritz et al. 2014b; Andrews et al. 2016). Care should be taken in adapter design and bioinformatics analysis to eliminate PCR duplicates (Schweyen et al. 2014; Tin et al. 2015), to account for indels in de novo assembly of RAD-tags (Puritz et al. 2014a), and to interpret data in

the context of potential biases due to ascertainment (Gautier et al. 2012; Arnold et al. 2013). The GBS or double-digest method does not include a random shearing step, and both ends of sequenced fragments are flanked by restriction enzyme cut sites. Reads from the same GBS locus, while potentially containing different alleles, will therefore always have the same start and end genome coordinates. Despite some differences between the different methods, RAD-based sequencing approaches capture segments of DNA from the genome and are generally considered to be reproducible. It is therefore a particularly useful and cost-effective method for studying relatedness and population structure in a large number of samples. Plenty of examples can be found in the literature where RAD-Seq methods have been used effectively to characterise patterns of neutral and adaptive genetic variation among species populations from marine environments (Hess et al. 2013; Benestan et al. 2015; Blanco-Bercial and Bucklin 2016; Lal et al. 2016). However, because RAD-Seq only samples a

small proportion of the genome, it is unlikely to characterise adaptive variation for species with low levels of linkage disequilibrium.

A third, less common method for reduced representation genome sequencing is sequence or target capture. Sequence capture allows for parallel enrichment of one to several thousand target sequences in a single experiment (Jones and Good 2015). There are several options available, most of which have been compared in the context of humans (Cummings et al. 2010; Mamanova et al. 2010; Sulonen et al. 2011; Samorodnitsky et al. 2015). This method depends on the investigator supplying a list of oligos probes for the sequence of interest, which means that genomic resources (such as exome sequences from RNA-Seq or long reads from PacBio SMRT sequencing) are a necessary first step for probe design. In some cases, genomic resources from closely related species may be used to design probes (Bi et al. 2012; Jones and Good 2015; Pavy et al. 2015). Sequence capture has the

### Box 1 Statistical issues in genome scans for invasions: intrinsic power and calculation of $p$ values

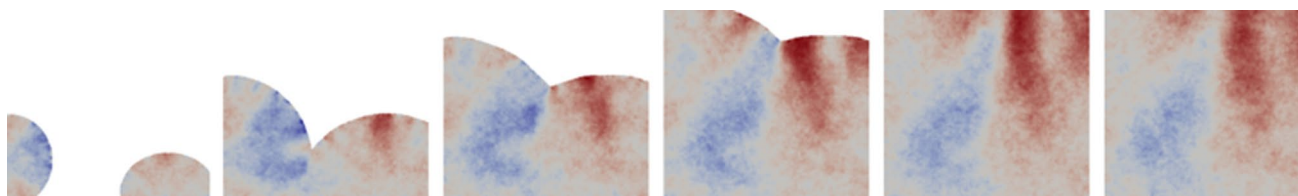
The statistical issue of intrinsic power is particularly acute in the context of marine invasions that leave a strong demographic signal in the genome. In the initial stages of invasion, the bottleneck of a few founding individuals will result in low genetic diversity for the founding population (Nei et al. 1975; Tajima 1989a, b; Tajima 1996). During the bottleneck, some genetic diversity indices like nucleotide diversity and heterozygosity are less affected, while a relative reduction in rare alleles will result in a positive value of Tajima's  $D$  (Tajima 1989a)—a pattern that is also results from balancing selection, but in this case results from neutral processes. If multiple invasive populations are founded by a few source populations, the finite number of populations in the data will increase the variance of the  $F_{ST}$  distribution. Genetic drift will dominate due to the small population sizes, which will increase the mean in the  $F_{ST}$  distribution.

Following the bottleneck, the invasive population size grows rapidly and this may create some signals in the genome that resemble selection. On average, alleles were at intermediate frequency before the bottleneck and will be rare after the bottleneck, and there will be a dearth of intermediate alleles in the expanding population. On average, there will be more rare variants than intermediate variants and this drives Tajima's  $D$  to be negative (Tenaillon et al. 2004)—a pattern that is also indicative of a selective sweep or purifying selection, but in this case results from neutral processes.

As the invasion proceeds, rapid expansion of an invasive lineage can result in allele surfing on the range front (Edmonds et al. 2004; Klopstein et al. 2006; Excoffier and Ray 2008). Allele surfing occurs at the expanding edge of the invasion, where population size is low. Due to the low  $N_e$ , genetic drift will dominate and alleles will be constantly drifting (or “surfing”) to high frequency and over a large area on the range edge (see Fig. 1). This signal is eroded by migration as more individuals migrate into the range front, thus resulting in large or clinal differences in neutral allele frequencies between more established populations and the invasion edge (Fig. 1). An allele that surfs to high frequency in the metapopulation may show a signal of a hard or soft sweep in the sample and may have an above average  $F_{ST}$ . Thus, allele surfing can reduce the intrinsic power of genome scans to detect selection because it results in some neutral loci with high genetic differentiation among populations, and it may also reduce the intrinsic power of a genetic–environmental association if it results in strong correlations between neutral loci and an environmental cline.

In addition to intrinsic power of the statistical test, there is the statistical issue of calculating significance of the test statistic (e.g. calculation of  $p$  values used to infer “outliers”). Calculating accurate  $p$  values requires controlling accurately for population demography, but unfortunately population demography is rarely known with accuracy. The most widely cited  $F_{ST}$  outlier methods—*fdist*/LOSITAN (Beaumont et al. 1996) and Bayescan (Foll and Gaggiotti 2008)—assume an island model or an isolation with migration model, respectively. The consequence of assuming an island model or the isolation with migration model under range expansion is that they result in a null distribution with a much narrower variance than the distribution of neutral alleles, thus resulting in many neutral loci with low  $p$  values (i.e. many false positives). In some cases, this problem may be alleviated by re-calibration of the  $p$  values to control for inflation (François et al. 2015). Newer statistics have been developed for genetic differentiation outlier tests (as well as genetic–environmental associations) and would be more relevant to apply to invasive species because they do not make specific assumptions about the population demography, but rather use the data to control for population structure. For example, Bayenv2 (Günther and Coop 2013) and BayPass (Gautier 2015) use a covariance matrix of allele frequencies, FLK (Bonhomme et al. 2010) uses a population tree, PCAdapt (Duforet-Frebourg et al. 2014) uses principal components, and OutFLANK (Whitlock and Lotterhos 2015) uses maximum likelihood based on a trimmed  $F_{ST}$  distribution.

Despite these potential limitations, genome scans can be a powerful approach towards understanding the role of adaptation in the invasion process. Investigators should take care to interpret the results, however, in the context of the potentially complex demographic history of the invasion.



**Fig. 1** Example of allele surfing for a single locus from a two-refugia simulation in Lotterhos and Whitlock (2014). *Blue colours* indicate low frequencies of the focal allele, and *red colours* indicate high frequencies of the focal allele. In this example, the refuge on the left experienced some surfing of the alternate allele early in expansion,

while the refuge on the right experienced extreme surfing of the focal allele during expansion. Although this was a neutral allele, allele surfing caused this locus to have higher than average  $F_{ST}$  on the landscape

advantage of improving detection of SNPs, indels, and highly variable regions, although repetitive structural variation (satellite DNA or transposable elements) and paralogs (duplicated genes) may still confound alignment and subsequent ascertainment (Novák et al. 2010; Miga 2015; Platt Ii et al. 2016). If probes are designed to reflect the full range of evolutionary rates of all loci (i.e. functional and non-functional sequences over a range of mutation rates, recombination rates, and background selection), sequence capture data should not suffer from the potential biases of RNA-Seq and the potential sparsity of RAD-Seq. Target capture methods are most appropriate for taxonomic groups with ample genetic resources that provide a suitable basis for probe development (Bourret et al. 2013; Deagle et al. 2013; Rosani et al. 2014).

An alternative to obtaining sequencing information at the individual level is to pool all or a subset of individuals from a treatment or population for genomic sequencing in order to obtain allele frequency estimates for the population (Futschik and Schlotterer 2010; Karlsen et al. 2013; Guo et al. 2016). Note that this method usually requires at the minimum a draft genome assembly (or a closely related reference) for aligning the reads. There are two approaches towards partial or whole-genome pool-seq: (1) an equal amount of DNA from each individual is added to the pool tagged with an adapter (each pool has a different barcode) (Schlötterer et al. 2014), or (2) each individual is barcoded and sequenced to low  $1 \times -2 \times$  coverage (Buerkle and Gompert 2012). The number of pools or individuals that can be sequenced in a single Illumina lane will depend on genome size. Both approaches are used to estimate allele frequencies for the pool (as in Schlötterer et al. 2014) or population (as in Buerkle and Gompert 2012). Note that the pooling process introduces a considerable amount of unknown variability in sampling that may bias estimates of allele frequencies, such as variability in the coverage among individuals. Thus, sampling design should be carefully considered based on the best practices (Buerkle and Gompert 2012; Schlötterer et al. 2014). Alignment of partial or whole-genome pool-seq data to closely

related species has not been well evaluated, and it is likely that important genomic features are lost in alignment. For example, the percentage of transposable elements identified in mammals using a database of human transposable elements declines drastically over 90 million years since divergence (Platt Ii et al. 2016). Since many marine invaders do not yet have sequenced genomes, whole-genome sequencing with alignment to related species should be approached with caution.

### Inferring the genetic basis of adaptation in the invasion process

There are four common statistical approaches used to identify the genetic basis of adaptation from genomic data: genotype–phenotype associations (more commonly known as genome-wide association studies or GWAS), differentiation outlier methods (commonly called  $F_{ST}$  outlier tests, but several related statistics have been proposed, see below), genetic–environmental associations (reviewed in Rellstab et al. 2015), and scans for selective sweeps (reviewed in Vatsiou et al. 2015; Schlamp et al. 2016; Stephan 2016). All of these approaches, commonly referred to as genome scans for “outliers”, seek to identify loci that exhibit patterns that are significantly different from the rest of the loci in the genome. Since a majority of the loci in the genome are presumed to be neutral, significant outliers are presumed to be under selection. These four major approaches have different strengths and weaknesses and may require different experimental designs.

Genotype–phenotype associations estimate the magnitude of the mutational effect different alleles have on the measured phenotype. The experimental design requires that a large number of individuals are phenotyped in a common garden experiment to minimise the effect of environmental differences among populations on phenotypes, and the statistical analysis requires that relatedness among those individuals is controlled for to minimise false associations (de Villemereuil et al. 2015). Thus, this approach

would not be possible with pooled sequencing approaches (unless the phenotype was discrete) and also requires high-density SNP data, especially in individuals sampled from natural populations with low levels of linkage disequilibrium. A similar, more widely used approach is to perform quantitative trait loci (QTL) mapping with RAD-tags on F1 or F2 families: a linkage map can be constructed without genomic resources to identify genomic region(s) underlying divergent traits in the parents [the linkage map can later be integrated with a whole-genome assembly, see Fierst (2015)]. Because of family structure in the data, lower density of SNP data can be used, but the resolution to determine the specific genomic region is also lower. Recently, the power of the QTL approach to uncover the genetic basis of body weight was demonstrated on a single generation of a sea cucumber species using two parents and 100 offspring without a whole-genome reference (Tian et al. 2015). Both genotype–phenotype associations and QTL mapping have the advantage of identifying loci that affect the phenotype, but outliers in this approach are not necessarily acted upon by selection: these are loci that affect the phenotype, and only field studies can demonstrate selection on that phenotype, or other scans for selection can demonstrate selection on that locus.

Differentiation outlier methods seek to identify loci that are overly differentiated when compared to the average genetic differentiation among loci in the genome, while genetic–environmental associations seek to identify loci that are significantly associated with an environmental variable (Hess et al. 2013; Milano et al. 2014; Berg et al. 2015; Guo et al. 2016). The experimental design requires that a large number of individuals are collected from populations on the landscape that have putatively adapted to local conditions (and for genetic–environmental associations, also a measure of the biotic or abiotic environment of each individual or population), and requires dense sampling of the genome to identify the targets of selection (Tiffin and Ross-Ibarra 2014). A widely used statistic for outlier differentiation methods is  $F_{ST}$ , a statistic that measures the variance in allele frequencies among populations compared to the mean allele frequency in all populations (Lotterhos and Whitlock 2014). When an allele exhibits local adaptation because of divergent or diversifying selection—that is, it is selected for in one population and selected against in another population—theory predicts that this allele will have a higher  $F_{ST}$  compared to the rest of the genome, all else being equal (see caveats below). While loci experiencing global balancing selection will have a low  $F_{ST}$  (because they have similar allele frequencies across populations), using  $F_{ST}$  to test for balancing selection is not advisable for two reasons: (1) loci with low sample sizes will have lower  $F_{ST}$  because of the correction for sample size in the estimate (Weir and Cockerham

1984), and (2) simulation studies have shown that tests for balancing selection have many false positives and low power (Foll and Gaggiotti 2008; Lotterhos and Whitlock 2014). More recently, a number of statistical tests have been developed for both differentiation outliers and genetic–environmental associations (reviewed in Hoban et al. 2016, in press).

The fourth major approach is to scan the genome for signatures of selective sweeps, or shifts in the frequency of a haplotype (regardless of the pattern of hitchhiking signal) within or among populations (Pritchard and Di Rienzo 2010). Classical sweep models typically detect dramatic haplotype frequency changes. In the context of marine invasions, selective sweep analyses can aid in determining whether a single adaptive de novo mutation arose on all haplotypes in the invasive lineage (hard sweep), or whether multiple haplotypes arose during the invasion process. The latter situation is known as a soft sweep and may occur when a *single* beneficial mutation arises from standing genetic variation on different haplotypes in multiple geographic locations or when *different* beneficial mutations arise (either de novo or from standing variation) to solve the same problem in multiple geographic locations (reviewed in Messer and Petrov 2013). Understanding the relative roles of selection from new mutation or from standing genetic variation is of central importance to our understanding of invasion success. For instance, adaptation during invasions is predicted to rely more often on standing genetic variation (generating soft sweeps) rather than on de novo mutation (generating hard sweeps), with some recent empirical support from plant systems (e.g. Vandepitte et al. 2014). The application of sweep methods to marine species has been limited, likely because many of them (1) require phased haplotype data and (2) require long sequences in order to calculate haplotype length. As an allele sweeps to fixation, nearby neutral loci will “hitchhike” along with it and studying the frequency distribution and length of haplotypes can elucidate the type of sweep (Messer and Petrov 2013). When data are available, there are two kinds of statistical approaches for detecting sweeps: those that use only a single population and those that use multiple populations. Methods that incorporate multiple populations, such as XP-EHH (Sabeti et al. 2007) and hapFLK (Fariello et al. 2013), may be particularly useful in the context of invasions, because the native population can be used as an outgroup to understand the ancestral distribution of haplotypes.

However, caution should be applied when interpreting results from the approaches discussed above, because rapid range expansion creates signatures at neutral loci that resemble selection (Box 1). In addition, there are two statistical issues that are relevant when applying these tests: the first is the intrinsic power of the statistic to separate

neutral from selected loci; the second is the ability of the method to accurately calculate  $p$  values for the test statistic (Box 1). The former issue will arise when population demography obfuscates or causes false signatures of signals of selection, while the latter issue arises when population demography is not accurately corrected for in the test statistic (such that the null distribution assumed by the statistical test has lower variance than the empirical distribution of neutral loci). These issues are described in more detail in Box 1.

These statistical issues suggest that a landscape-genomic study will have highest power to detect genetic signals of adaptation in the native range and low power in the invasive range. Therefore, some of these statistical issues may be overcome by testing whether candidate loci from the native range, identified by a landscape-genomic study, are associated with fitness in experiments in the invasive range. One approach is to grow the offspring of individuals collected from different environments in a common garden experiment and statistically test whether genotypes at candidate SNPs are significantly associated with a measure of fitness such as growth or fecundity. While this approach has yet to be applied to marine invasive species, for a good demonstration of this approach in the plant *Medicago truncatula* see Yoder et al. (2014). Another approach is to raise a large number of offspring in one or more environments that will cause some mortality, and measure the differential survival of alleles as a change in allele frequency between the beginning and end of the experiment (Pespeni et al. 2013; Lotterhos and Schaal 2014; Soria-Carrasco et al. 2014). This approach assumes that individuals bearing an advantageous allele have an increased probability of survival. One could then statistically test whether a priori candidate genes, identified in the native range, exhibit statistically significant allele frequency changes. Whether it will be practical to carry out these experimental approaches for many marine species may be the main limitation because they are hard to rear in the laboratory, but if offspring can be generated and raised for a short period of time, then these approaches may be feasible. Comparing different experimental designs in the context of realistic invasion histories would be a fruitful area for future simulation studies.

### The use of RNA-based genomic approaches for understanding marine invasions

Transcriptome-based analyses are an increasingly standard approach to population genomics, particularly in non-model systems such as marine species (De Wit et al. 2015). The transcriptome is the full complement of RNA molecules (transcripts) within a cell, reflecting actively

expressed genes at a given point in time (Lu et al. 2013). These transcripts encompass both protein-coding and non-coding RNA, along with their alternatively spliced, polyadenylated, and RNA-edited forms (Moreton et al. 2016). By targeting RNA, much of the power of genomic approaches can be applied to systems with no pre-existing genetic resources (Gayral et al. 2013), enabling genome-wide analyses of nearly any species including non-model marine invasive species.

The primary high-throughput approach to this is RNA-Seq, which works by isolating either total RNA or a subset enriched for specific classes of RNAs, including mRNA and miRNA, and sequencing it on a range of high-throughput platforms. Since RNA-Seq can generate both sequence (SNP) and gene expression data, it can be applied to a range of questions central to invasion success. Changes in gene expression may arise from both plasticity and local adaptation in response to novel environmental conditions experienced during the invasion process. Lately, there has been increasing support for the idea that selection acting on the regulation of gene expression can drive local adaptations (Fraser et al. 2010; Fraser 2011, 2013; Larsen et al. 2011; Metzger et al. 2016). Given that regulatory changes may act faster, and more likely influence gene expression than protein-coding changes (Fraser 2013), these molecular mechanisms may be especially relevant to adaptation during marine invasions. The application of RNA-Seq experiments can be used to investigate these particular molecular responses. Larsen et al. (2011) provide four such comparative experimental frameworks for variation in gene expression: (1) within populations exposed to different environmental conditions—revealing environmental plasticity, (2) within populations exposed to the same environmental conditions—explaining life-history variation, (3) between populations exposed to common garden conditions, or reciprocal transplantation, to test for a genetic basis for gene expression variation, and (4) within and across species to identify signals of adaptive divergence.

With thoughtful experimental design, expression data can complement and enhance sequence data to give more nuanced insights into the process of adaptation. While changes in coding gene sequence can have dramatic impacts on organismal fitness (Hochachka and Somero 2002; Fields et al. 2006), it is becoming increasingly clear that regulatory changes play a significant role in adaptation (Larsen et al. 2011; Romero et al. 2012; Fraser 2013). Although RNA-Seq will not capture non-coding promoter regions that may be key for gene regulation, expressed proteins like transcription factors that bind to these promoter regions will be captured. In the marine realm, several recent studies have pointed to adaptive expression changes as being key to local

adaptation in fishes (Whitehead and Crawford 2006; Larsen et al. 2007). Given the important role regulatory evolution may play in local adaptation, this process may be particularly relevant to invasive species, of which many marine invasions could serve as excellent models for future investigations.

Sequencing reads from RNA-Seq can be mapped back onto either an existing genome/transcriptome or assembled de novo (De Wit et al. 2015; Richardson and Sherman 2015). Recently, a number of analytical pipelines and “best practices” guidelines have been developed for de novo assembly and transcriptome analysis, facilitating its broader use in ecological and evolutionary studies (De Wit et al. 2012, 2015; Gayral et al. 2013; Moreton et al. 2016). However, transcriptome-derived markers differ from their genome-derived counterparts in several important respects. First, they represent a more selective subset of the genome, since only markers in expressed genes can be identified, so important non-coding regulatory changes or genes expressed in unsampled tissues will not be captured. However, careful experimental design can provide gene expression data to help explore regulatory adaptation (e.g. Whitehead and Crawford 2006; Wray et al. 2013). In addition, many RNA-derived markers are not anonymous. Instead, they are associated with specific known genes based on transcriptome annotation. This context can be particularly useful as it permits functional analyses of selection, and provides specific targets for future work (Morozova and Marra 2008; Limborg et al. 2012; De Wit and Palumbi 2013).

Careful consideration must be given to the application of these experiments to avoid potential confounding factors when looking for signatures of local adaptation. For example, developmental plasticity, maternal, and epigenetic effects (discussed below)—which arise from the interplay of environmental, parental, and genetic background variability—may complicate the interpretation of gene expression (Larsen et al. 2011). Multigenerational common garden experiments may help to resolve some of these issues; however, they can be challenging for non-model systems. Yet, many marine invasive species lend themselves to such experiments. Furthermore, targeting the larval stages of these species, particularly of marine invertebrates, has a number of distinct advantages: (1) it is one of the most vulnerable life-history stages and therefore likely to respond strongly to selection (Richardson and Sherman 2015), (2) the entire organism can be sampled negating the issue of which tissue to target for analysis, and (3) this stage can be targeted for controlled laboratory experiments as many species can be induced to spawn in the laboratory and produce numerous offspring (Byrne et al. 1997; Fletcher and Forrest 2011; Pespeni et al. 2013; Sherman et al. 2015; Zhan et al. 2015). While

these controlled laboratory experiments lend themselves towards analysing differential expression, inferring allele frequencies from RNA-Seq on pools of individuals should be approached with caution because allele-specific expression could bias allele frequency estimates (for an example on how to deal with this issue see Pespeni et al. 2013).

To date, few studies have investigated how transcriptomic changes might reveal adaptation in a marine invasion context. Lockwood et al. (2010), using microarrays, revealed that differences in the transcriptional response to heat stress between invasive (*Mytilus galloprovincialis*) and native (*M. trossulus*) blue mussel species might explain the former’s invasive success. Yet, differences in the response to salinity stress between the same two species were subtle (Lockwood and Somero 2011). Another study using SNPs identified by RNA-Seq in the European green crab (*Carcinus maenas*) found that local adaptation has likely played a significant role in structuring native range populations and may have influenced the species’ invasion dynamics (see Box 2). More broadly, transcriptome analyses (both gene expression and sequence variation) have provided important insights into the responses of non-model marine organisms to environmental changes including salinity fluctuations, osmotic stress, ocean acidification (Todgham and Hofmann 2009; Larsen et al. 2011; Lv et al. 2013; Pespeni et al. 2013; Hui et al. 2014; Zhao et al. 2014), thermal stress (Gracey et al. 2004; Stillman and Tagmount 2009; Franssen et al. 2011; De Wit and Palumbi 2013; Harms et al. 2014; Gleason and Burton 2015), and temperature gradients (Whitehead and Crawford 2006). We have also recently seen the generation of several transcriptomic resources for marine invasive species (Uliano-Silva et al. 2014; Richardson and Sherman 2015; Tepolt and Palumbi 2015; Verbruggen et al. 2015) that will hopefully lead to a proliferation of research in this area.

While transcriptomic approaches have many benefits, there are some limitations. RNA-Seq results are sensitive to tissue type, sampling conditions, life stage of the organism, and any number of other factors, some of which may not be predictable in advance (Gracey et al. 2004; Scott and Johnston 2012; Lenz et al. 2014; Verbruggen et al. 2015). Furthermore, a single snapshot in time of intermediate mRNA forms may not correlate with the final abundance of proteins because of post-translational modification and RNA silencing (Feder and Walser 2005). Also, the inclusion of biological replicates is fundamental to gene expression studies; they directly influence the power to detect expression differences between treatments, more so than increasing the amount of sequencing per individual (Liu et al. 2014).

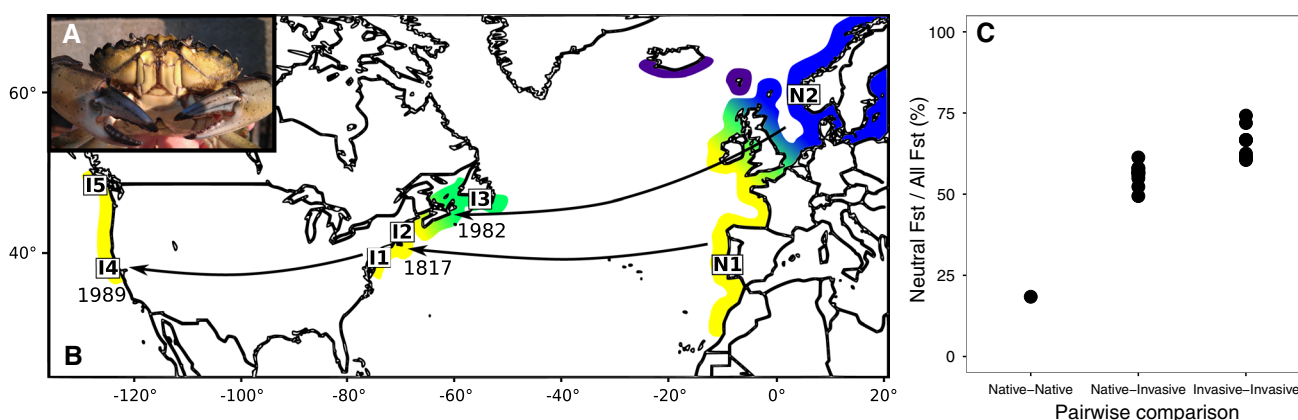
Assembly, annotation, and data analysis for non-model marine invaders also present a significant challenge. The process of de novo transcriptome assembly can be analytically complicated for understudied systems. While it is unlikely that a truly complete representation of the transcriptome can be currently assembled, there are methods for the assessment of transcriptome completeness and

**Fig. 3** A heat map of the 621 differentially expressed genes between range core (dark and light blue circles) and range edge (brown and orange circles) invasive cane toads (*Rhinella marina*) in Australia, based on RNA-Seq count data from Rollins et al. (2015). The log<sub>2</sub> fold change in gene expression (rows) by sample (column) is shown; red is upregulated, green down. The inset map of northern Australia indicates the location of sampling sites, as well as the distribution of cane toads in northern Australia (area to the east of the black line)

## Box 2 RNA-Seq approaches to understanding marine invasions

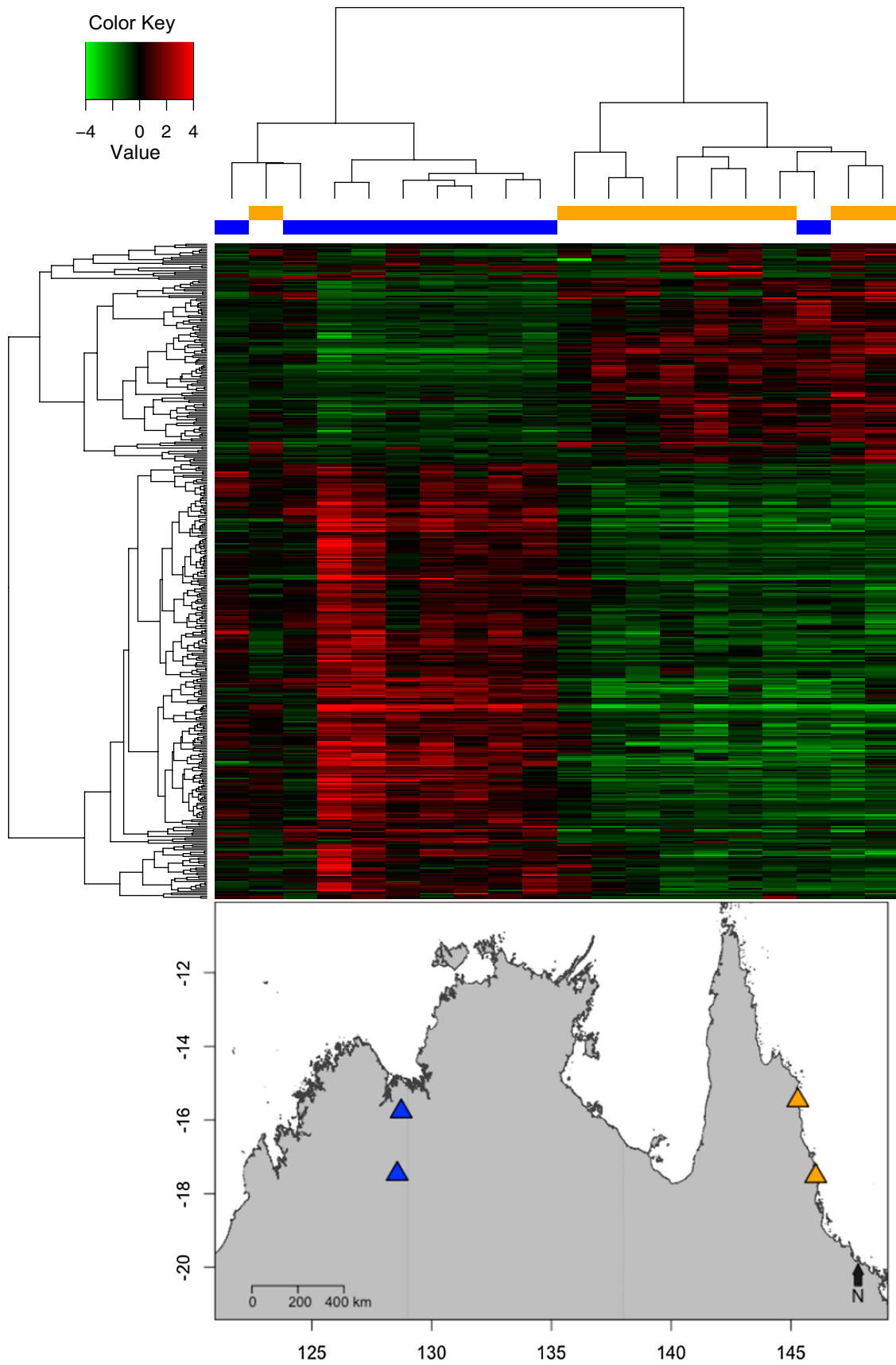
The application of RNA-Seq experiments to marine invasions provides exciting opportunities to examine evolutionary mechanisms during the invasion process. Yet, to date, we are aware of only one study doing so. This study used transcriptome-derived SNPs to assess the relative contributions of adaptive and neutral processes in the spread of the European green crab (*Carcinus maenas*) in North America (Tepolt and Palumbi 2015). The species' invasion history in this region is complex (Fig. 2), potentially related to local adaptation to temperature in the native range (Roman 2006). Physiological data supported this hypothesis, demonstrating significant and parallel variation in heat and cold tolerance within both the native and invasive ranges (Tepolt and Somero 2013). Tepolt & Palumbi (2015) sampled seven populations across thermal gradients in the native and North American invasive ranges, generating a panel of 10,809 SNPs. They found significant structure between populations and clear signatures of the species' varied invasion history. By comparing overall differentiation with that derived from a putatively neutral subset of SNPs, they demonstrated that neutral markers accounted for only 20 % of the variance in allele frequencies among subpopulations ( $F_{ST}$ ) in the native range (Fig. 2C). In contrast, neutral markers explained a much larger proportion of the variance in allele frequencies among subpopulations in the invasive range, likely reflecting the role of drift and related processes in structuring non-equilibrium invasive populations. They concluded that native range structure was maintained primarily by selection, suggesting that invasion risk and persistence may vary within the species and that this native range adaptation likely shaped the observed invasion dynamics.

In terms of gene expression, we are unaware of an example using RNA-Seq in a marine invasion context, although there are microarray examples (mentioned previously). We provide the following terrestrial example to illustrate the application of this approach to evolutionary questions during a species invasion. A recent study by Rollins et al. (2015) compared gene expression variation within the cane toad (*Rhinella marina*) invasion in Australia. Cane toads have expanded their range across tropical and subtropical Australia (Urban et al. 2008) at an increasing rate (Phillips et al. 2006), leading Rollins et al. (2015) to suggest that gene expression divergence across the range may be linked to increased dispersal ability at the invasion front. By comparing expression from muscle tissue between individuals from two range core and two edge populations, they identified 479 upregulated and 142 downregulated transcripts on the range edge (Fig. 3). Several transcripts upregulated at the edge encode mitochondrial matrix enzymes (including *KLF15* and *BCAT2*) that are involved in maintaining muscle mass. They also found upregulation of genes involved in cellular repair, along with an overrepresentation of gene ontology terms linked to upregulated genes involved in energy production and the response to oxidative stress, at the range edge. The upregulation of these genes indicates increased activity of cane toads and may underpin the dispersal success of range edge individuals (Rollins et al. 2015). The authors do note one important caveat that the differences seen between the core and edge may also reflect environmental as well as genetic effects and suggest that the application of a common garden experiment is a critical next step in disentangling the two.



**Fig. 2** a The European green crab (*Carcinus maenas*). b Green crab invasion history and genetic structure in Europe (native range) and North America (invasive range). Arrows indicate invasion events, with dates of first record (Carlton and Cohen 2003). Colours give a cartoon indication of genetic background, based largely on 10,809 mRNA-Seq-derived SNPs and supplemented by broader geographic sampling

with COI and microsatellites (Roman and Palumbi 2004; Roman 2006; Darling et al. 2008; Tepolt and Palumbi 2014, 2015). Boxed codes are sampling sites from Tepolt and Palumbi (2015): N Native range, I Invasive range. c Proportion of overall pairwise differentiation at 10,809 SNPs captured by a putatively neutral subset, classed by native and invasive status of each pairwise population comparison



quality (reviewed in Moreton et al. 2016). Functional annotation of assembled sequences (contigs) remains a significant hurdle because the extensively utilised BLASTX annotation procedure—whereby assembled contigs are matched to available protein databases to identify coding genes—is limited to taxonomically biased genomic resources (Driskell et al. 2004; Riesgo et al. 2012; GIGA Community of Scientists 2014). For this reason, taxonomically distinctive genes may remain unannotated, which is especially true for assembled non-coding RNAs where the available resources are sparse although there are now databases (e.g. Jurka et al. 2005; Derrien et al. 2012; Quek et al. 2015) and new methods available (Koskinen et al. 2015; Sun et al. 2015) that can aid in the annotation of non-coding RNAs. Inference of the biological function of annotated contigs is often made utilising gene ontology (GO) annotations (e.g. Richardson and Sherman 2015; Rollins et al. 2015). While these functional GO classifications are based on computational or experimental evidence in model species, the specific function of the gene may not translate across taxonomically distinct groups. Finally, the application of analytical methods is also limited for RNA-Seq data. For example, statistical analyses that require long sequences, such as sliding window and selective sweep methods, cannot be applied because transcripts are typically too short and the physical location of sequences on chromosomes is unknown. This constrains many RNA-based studies of adaptation largely to outlier approaches or genetic–environmental associations, which may not perform ideally in the distinctive dynamics of invasive populations (Box 1). Notwithstanding these important caveats, RNA-Seq remains an exciting methodology for investigating aspects of the invasion process in non-model marine invasive species.

### The role of epigenetics in invasion success

Recently, the importance of epigenetic variation in ecology and evolution has gained considerable attention (Vanderhoeven et al. 2010; Schrey et al. 2012; Mendizabal et al. 2014; Metzger and Schulte 2016). Epigenetic variation results from heritable (meiotically or mitotically) chemical modifications to DNA that may affect gene expression but do not alter the underlying nucleotide sequence. Examples include methylation, non-coding RNA and histone modifications. Epigenetic variation can be environmentally induced (Angers et al. 2010) and, in some cases, has been shown to be trans-generationally heritable (Rakyan et al. 2003; Crews et al. 2007). However, in some taxa (e.g. mammals), epigenetic marks are mostly cleared during development (Morgan et al. 2005), preventing trans-generational inheritance. However, when marks are not transmitted across

generations, a rapid shift in epigenetic marks resulting from environmental input may allow organisms to buffer the effects of environmental stress, making this a particularly important ecological process determining the success of invasive species (Angers et al. 2010).

It has been suggested that heritable epigenetic variation may be an important mechanism by which invasive species adapt to novel environments, especially when genetic variation is low or absent (Pérez et al. 2006; Angers et al. 2010; Kilvitis et al. 2014), and this idea is supported by empirical evidence in widely varying taxa (e.g. plants and birds) (Richards et al. 2012; Liebl et al. 2013). Population bottlenecks often seen in introductions may even stimulate epigenetic variation, resulting in novel phenotypes on which selection may then act (Rapp and Wendel 2005). Additionally, DNA methylation itself may introduce DNA nucleotide variation because methylated nucleotides have higher mutation rates than unmethylated nucleotides (Rideout et al. 1990). As further evidence to the idea that methylation can assist evolution, methylation levels are correlated with evolutionary rates across the great tit genome (Laine et al. 2015). These lines of evidence suggest that epigenetic variation may help to explain the “invasion paradox”, where introduced populations are able to adapt to novel environments, despite low genetic diversity (Allendorf and Lundquist 2003; Pérez et al. 2006; Rollins et al. 2015).

Studies of epigenetic variation in non-model (ecological) systems are almost exclusively limited to investigations of DNA methylation, primarily because of cost and accessibility. The most common DNA methylation detection method used is methylation-sensitive amplified fragment length polymorphism (MS-AFLP). This method does not require a reference genome, but is an anonymous approach and can only assay methylation at the cut site of chosen restriction enzymes. Alternatively, methods employing sodium bisulphite sequencing are available, but, previously, the analysis of these data required reference genomes, making such approaches untenable for non-model organisms. Quite recently, a number of reduced representation sequencing methods have been developed to assay genome-wide methylation that do not require the use of a reference genome (Verhoeven et al. 2016 and references therein). A thorough review of approaches used to measure methylation has recently been published (Metzger et al. 2016).

DNA methylation is common across life forms, but the patterns and degree of methylation vary widely across taxa (Angers et al. 2010). For example, methylated cytosines have not been detected in *Caenorhabditis elegans*; *Drosophila melanogaster* have low levels of methylated cytosines; other invertebrates have large amounts of methylated DNA (Bird 2002); vertebrates have high levels of

methylation throughout their genomes; and methylation is ubiquitous in plants (Colot and Rossignol 1999). In vertebrates, most methylation occurs at CpG di-nucleotides distributed throughout the genome, with the exception of CpG islands in promoter regions of genes. In contrast, some invertebrates carry “mosaic” blocks of methylated and unmethylated sites across their genomes (Angers et al. 2010). Our knowledge of methylation in fish has been led by work on zebrafish; this research and emerging epigenetic work on marine species indicates that methylation patterns in fish are similar to those of other vertebrates, although it is less clear whether epigenetic marks are reset during development as they are in mammals (reviewed in Metzger and Schulte 2016). In addition to taxon-specific variation in DNA methylation, levels of methylation can also vary considerably within species (Eichten et al. 2011; Massicotte et al. 2011).

Despite recent advances in this field, epigenetic effects in natural populations of all taxa remain largely unknown (Richards et al. 2012; Verhoeven et al. 2016) and few invasive species have been investigated to determine the importance of epigenetics during the invasion process. Methylation patterns have been characterised in a number of marine organisms including the Pacific oyster (*Crassostrea gigas*; Gavery and Roberts 2010; Rivière 2014), the pearl oyster (*Pinctada fucata*; Li et al. 2015), sea urchins (*Echinus esculentus*; Bird et al. 1979), sea squirts (*Ciona intestinalis*; Simmen et al. 1999), Zhikong scallop (*Chlamys farreri*; Sun et al. 2014), Chinese shrimp (*Fenneropenaeus chinensis*; He et al. 2015), the common octopus (*Octopus vulgaris*; Díaz-Freije et al. 2014), sea lampreys (*Petromyzon marinus*; Covelo-Soto et al. 2015), algae such as *Ulva reticulata* (Gupta et al. 2012), and plants such as *Posidonia oceanica* (Greco et al. 2011). To our knowledge, there have been no specific studies characterising the epigenome of invasive marine species to understand the role of epigenetics during the invasion processes. Nevertheless, there have been a limited number of epigenetic studies on terrestrial invasive taxa. The first study of DNA methylation in an invasive animal population investigated sparrows (*Passer domesticus*) from two introductions and found a positive relationship between levels of DNA methylation and introduction age (Schrey et al. 2012). Liebl et al. (2013) also investigated methylation in this species and found a significant negative relationship between genetic and epigenetic (DNA methylation) diversity, suggesting that epigenetic variation may compensate for loss of genetic variation in introductions. In a selection experiment investigating simulated spatial sorting in the two-spotted spider mite (*Tetranychus urticae*), evidence of the evolution of dispersal behaviour was attributed to trans-generational maternal epigenetic inheritance (Van Petegem et al. 2015). Spatial sorting has been identified as an important factor in many invasions including

amphibians (Shine et al. 2011), birds (Berthouly-Salazar et al. 2012), and insects (Lombaert et al. 2014), yet the spider mite study is the first to link spatial sorting to epigenetic change.

Our understanding of how epigenetics affects invasion ability remains sparse, but current evidence suggests that this avenue is worth investigating. It is well established that phenotypic plasticity can facilitate invasion, and it has been suggested that it may serve as a stepping stone that invasive species can use during the establishment phase, which may then be followed by evolutionary change (Baldwin 1896; Waddington 1953; Carroll 2008). Such trans-generational plasticity is likely to be underlain by epigenetic mechanisms. Presently, there is dearth of research investigating the interplay of evolution and plasticity (Waples 2016) or how plasticity mediates the effects of environmental change during invasion (Hendry 2015). Marine taxa may provide excellent models to further this field of research because the breeding systems of many lend themselves to large-scale manipulative experiments, where genetic and epigenetic effects can be separated, as can their impacts on evolution and plasticity.

## Concluding remarks

For many years, marine invasive species management has benefited greatly from genetic tools. These have provided much needed insights into species taxonomies, dispersal capacities, invasion pathways, reproductive biology, and demographic histories. The latest DNA- and RNA-based approaches afforded by NGS technologies now offer the opportunity to vastly improve our knowledge of pest biology and ecology by providing unprecedented sensitivity for resolving patterns of genetic structure. For the first time, these genomic tools provide the means to directly assess the roles of adaptive genetic variation and plasticity in the success of marine invasions. These represent significant gaps in our current understanding of marine invasive biology; however, genomic tools are expected to greatly assist the characterisation of adaptive variation within and among invasive and native populations, the identification of potential candidate genes underlying adaptive traits, and understanding the role of differential gene expression and epigenetic variation in plastic responses to novel environments. These “omic” approaches are increasingly used in a range of non-model organisms but have only been applied to a limited number of invasive species, and only a few from the marine environment. This will undoubtedly change over the coming years as the costs associated with NGS reduce and bioinformatic tools improve.

While high-density genome scans promise unprecedented resolution for addressing a range of ecological and

evolutionary questions, it is clear that strong demographic signals arising from the invasion process can lead to false signals of selection using current statistical approaches (Box 1). It is therefore important that researchers recognise these limitations and apply appropriate sampling designs and develop new analytical tools to ensure data are correctly interpreted. It should also be recognised that these tools should be used as a starting point for functional genomic analyses and need to be combined with traditional common garden, reciprocal transplant, and quantitative breeding experiments to validate the loci underlying important adaptive traits, or loci that appear to be responding to environmental variation. Many marine invasive species have life-history traits that make them particularly amenable for combining genomic approaches with quantitative laboratory experiments. Many invasive species are characterised by high levels of fecundity and external fertilisation that allow for replicated laboratory assays to assess the genetic basis of variable traits across environmental gradients. Additionally, many are capable of clonal reproduction allowing the same genotype to be exposed to different conditions to evaluate epigenetic and plastic responses to environmental variation. These complementary approaches provide an opportunity to streamline genetic studies of invasive species and tackle conservation issues in a more holistic fashion, by enabling population dynamics and adaptive potentials of pest species to be investigated in detail and simultaneously.

**Acknowledgments** Research support was provided by funding from the Centre for Integrative Ecology, Deakin University to CDHS, ADM, MFR, and LAR.

#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

- Ab Rahim ES, Nguyen TTT, Ingram B, Riginos C, Weston KJ, Sherman CDH (2016) Species composition and hybridisation of mussel species (Bivalvia: Mytilidae) in Australia. *Mar Freshw Res*. doi:10.1071/MF15307
- Allendorf FW, Lundquist LL (2003) Introduction: population biology, evolution, and control of invasive species. *Conserv Biol* 17:24–30
- Allendorf FW, Hohenlohe PA, Luikart G (2010) Genomics and the future of conservation genetics. *Nat Rev Genet* 11:697–709. doi:10.1038/nrg2844
- Andrews KR, Good JM, Miller MR, Luikart G, Hohenlohe PA (2016) Harnessing the power of RADseq for ecological and evolutionary genomics. *Nat Rev Genet*. doi:10.1038/nrg.2015.28
- Angers B, Castonguay E, Massicotte R (2010) Environmentally induced phenotypes and DNA methylation: how to deal with unpredictable conditions until the next generation and after. *Mol Ecol* 19:1283–1295
- Arnaud-Haond S, Candeias R, Serrao EA, Teixeira SJL (2013) Microsatellite markers developed through pyrosequencing allow clonal discrimination in the invasive alga *Caulerpa taxifolia*. *Conservation Genetics Resources* 5:667–669. doi:10.1007/s12686-013-9878-8
- Arnold B, Corbett-Detig RB, Hartl D, Bomblies K (2013) RADseq underestimates diversity and introduces genealogical biases due to nonrandom haplotype sampling. *Mol Ecol* 22:3179–3190. doi:10.1111/mec.12276
- Ayre DJ (1995) Localized adaptation of sea anemone clones. Evidence from transplantation over two spatial scales. *J Anim Ecol* 64:186–196
- Baldwin JM (1896) A new factor in evolution (Continued). *American naturalist* 30:536–553
- Bax N, Williamson A, Aguero M, Gonzalez E, Geeves W (2003) Marine invasive alien species: a threat to global biodiversity. *Mar Policy* 27:313–323. doi:10.1016/S0308-597X(03)00041-1
- Bayha KM, Chang MH, Mariani CL, Richardson JL, Edwards DL, DeBoer TS, Moseley C, Aksoy E, Decker MB, Gaffney PM, Harbison GR, McDonald JH, Caccone A (2015) Worldwide phylogeography of the invasive ctenophore *Mnemiopsis leidyi* (Ctenophora) based on nuclear and mitochondrial DNA data. *Biol Invas* 17:827–850. doi:10.1007/s10530-014-0770-6
- Beaumont MA, Nichols RA, Sciences PB, Dec N (1996) Evaluating loci for use in the genetic analysis of population structure. *Proc R Soc Lond B Biol Sci* 263:1619–1626
- Benestan L, Gosselin T, Perrier C, Sainte-Marie B, Rochette R, Bernatchez L (2015) RAD genotyping reveals fine-scale genetic structuring and provides powerful population assignment in a widely distributed marine species, the American lobster (*Homarus americanus*). *Mol Ecol* 24:3299–3315. doi:10.1111/mec.13245
- Benzie JAH, Ballment E, Chisholm JRM, Jaubert JM (2000) Genetic variation in the green alga *Caulerpa taxifolia*. *Aquat Bot* 66:131–139
- Berg PR, Jentoft S, Star B, Ring KH, Knutsen H, Lien S, Jakobsen KS, André C (2015) Adaptation to low salinity promotes genomic divergence in Atlantic Cod (*Gadus morhua* L.). *Genome Biol Evol* 7:1644–1663. doi:10.1093/gbe/evv093
- Berthouly-Salazar C, van Rensburg BJ, Le Roux JJ, van Vuuren BJ, Hui C (2012) Spatial sorting drives morphological variation in the invasive bird, *Acridotheris tristis*. *PLoS ONE* 7:e38145. doi:10.1371/journal.pone.0038145
- Bi K, Vanderpool D, Singhal S, Linderoth T, Moritz C, Good JM (2012) Transcriptome-based exon capture enables highly cost-effective comparative genomic data collection at moderate evolutionary scales. *BMC Genom* 13:403. doi:10.1186/1471-2164-13-403
- Bird A (2002) DNA methylation patterns and epigenetic memory. *Genes Dev* 16:6–21
- Bird AP, Taggart MH, Smith BA (1979) Methylated and unmethylated DNA compartments in the sea urchin genome. *Cell* 17:889–901
- 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. doi:10.1111/j.1472-4642.2010.00703.x
- Blanco-Bercial L, Bucklin A (2016) New view of population genetics of zooplankton: RAD-seq analysis reveals population structure of the North Atlantic planktonic copepod *Centropages typicus*. *Mol Ecol* 25:1566–1580
- Bock DG, Caseys C, Cousens RD, Ma Hahn, Heredia SM, Hübner S, Turner KG, Whitney KD, Rieseberg LH (2015) What we still don't know about invasion genetics. *Mol Ecol* 24:2277–2297. doi:10.1111/mec.13032
- Boehm JT, Waldman J, Robinson JD, Hickerson MJ (2015) Population genomics reveals seahorses (*Hippocampus erectus*) of the

- western Mid-Atlantic Coast to be residents rather than vagrants. PLoS ONE. doi:[10.1371/journal.pone.0116219](https://doi.org/10.1371/journal.pone.0116219)
- Bolte S, Fuentes V, Haslob H, Huwer B, Thibault-Botha D, Angel D, Galil B, Javidpour J, Moss AG, Reusch TBH (2013) Population genetics of the invasive ctenophore *Mnemiopsis leidyi* in Europe reveal source-sink dynamics and secondary dispersal to the Mediterranean Sea. Mar Ecol Prog Ser 485:25–U46. doi:[10.3354/meps10321](https://doi.org/10.3354/meps10321)
- Bonhomme M, Chevalet C, Servin B, Boitard S, Abdallah JM, Blott S, San Cristobal M, Sancristobal M (2010) Detecting selection in population trees: the Lewontin and Krakauer test extended. Genetics 186:241–262. doi:[10.1534/genetics.104.117275](https://doi.org/10.1534/genetics.104.117275)
- Booth D, Provan J, Maggs CA (2007) Molecular approaches to the study of invasive seaweeds. Bot Mar 50:385–396. doi:[10.1515/bot.2007.043](https://doi.org/10.1515/bot.2007.043)
- Borsa P, Daguin C, Bierne N (2007) Genomic reticulation indicates mixed ancestry in Southern-Hemisphere *Mytilus* spp. mussels. Biol J Linn Soc 92:747–754. doi:[10.1111/j.1095-8312.2007.00917.x](https://doi.org/10.1111/j.1095-8312.2007.00917.x)
- Bourret V, Kent MP, Primmer CR, Vasemägi A, Karlsson S, Hindar K, McGinnity P, Verspoor E, Bernatchez L, Lien S (2013) SNP-array reveals genome-wide patterns of geographical and potential adaptive divergence across the natural range of Atlantic salmon (*Salmo salar*). Mol Ecol 22:532–551. doi:[10.1111/mec.12003](https://doi.org/10.1111/mec.12003)
- Buerkle AC, Gompert Z (2012) Population genomics based on low coverage sequencing: how low should we go? Mol Ecol. doi:[10.1111/mec.12105](https://doi.org/10.1111/mec.12105)
- Burden CT, Stow AJ, Hoggard SJ, Coleman MA, Bishop MJ (2014) Genetic structure of *Carcinus maenas* in southeast Australia. Mar Ecol Prog Ser 500:139–147
- Byrne M, Morrice M, Wolf B (1997) Introduction of the northern Pacific asteroid *Asterias amurensis* to Tasmania: reproduction and current distribution. Mar Biol 127:673–685
- Carlton JT, Cohen AN (2003) Episodic global dispersal in shallow water marine organisms: the case history of the European shore crabs *Carcinus maenas* and *C. aestuarii*. J Biogeogr 30:1809–1820. doi:[10.1111/j.1365-2699.2003.00962.x](https://doi.org/10.1111/j.1365-2699.2003.00962.x)
- Carroll SP (2008) Facing change: forms and foundations of contemporary adaptation to biotic invasions. Mol Ecol 17:361–372
- Chao A, Jost L, Hsieh TC, Ma KH, Sherwin WB, Rollins LA (2015) Expected Shannon entropy and Shannon differentiation between subpopulations for neutral genes under the finite island model. PLoS ONE. doi:[10.1371/journal.pone.0125471](https://doi.org/10.1371/journal.pone.0125471)
- Cho YA, Kim E-M, Kim M-J, Kang J-H, Dong CM, An HS, An C-M, Park MA, Park JY (2014) A rapid and simple method for distinguishing two mitten crabs (*Eriocheir sinensis* and *Eriocheir japonica*) in Korea using PCR-RFLP and PCR. Food Control 36:20–23. doi:[10.1016/j.foodcont.2013.07.040](https://doi.org/10.1016/j.foodcont.2013.07.040)
- Colautti RI, Lau JA (2015) Contemporary evolution during invasion: evidence for differentiation, natural selection, and local adaptation. Mol Ecol 24:1999–2017. doi:[10.1111/mec.13162](https://doi.org/10.1111/mec.13162)
- Colot V, Rossignol J-L (1999) Eukaryotic DNA methylation as an evolutionary device. BioEssays 21:402–411. doi:[10.1002/\(SICI\)1521-1878\(199905\)21:5<402:AID-BIES7>3.0.CO;2-B](https://doi.org/10.1002/(SICI)1521-1878(199905)21:5<402:AID-BIES7>3.0.CO;2-B)
- Covelo-Soto L, Saura M, Morán P (2015) Does DNA methylation regulate metamorphosis? The case of the sea lamprey (*Petromyzon marinus*) as an example. Comp Biochem Physiol B Biochem Mol Biol 185:42–46
- Crews D, Gore AC, Hsu TS, Dangleben NL, Spinetta M, Schallert T, Anway MD, Skinner MK (2007) Transgenerational epigenetic imprints on mate preference. Proc Natl Acad Sci 104:5942–5946. doi:[10.1073/pnas.0610410104](https://doi.org/10.1073/pnas.0610410104)
- Cummings N, King R, Rickers A, Kaspi A, Lunke S, Haviv I, Jowett JBM (2010) Combining target enrichment with barcode multiplexing for high throughput SNP discovery. BMC Genom 11:641. doi:[10.1186/1471-2164-11-641](https://doi.org/10.1186/1471-2164-11-641)
- Dabe EC, Sanford RS, Kohn AB, Bobkova Y, Moroz LL (2015) DNA methylation in basal metazoans: insights from Ctenophores. Integr Comp Biol 55:1096–1110. doi:[10.1093/icb/icv086](https://doi.org/10.1093/icb/icv086)
- 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. doi:[10.1111/j.1365-294X.2008.03978.x](https://doi.org/10.1111/j.1365-294X.2008.03978.x)
- Darling JA, Tsai Y-HE, Blakeslee AMH, Roman J (2014) Are genes faster than crabs? Mitochondrial introgression exceeds larval dispersal during population expansion of the invasive crab *Carcinus maenas*. R Soc Open Sci. doi:[10.1098/rsos.140202](https://doi.org/10.1098/rsos.140202)
- Davey JW, Hohenlohe PA, Etter PD, Boone JQ, Catchen JM, Blaxter ML (2011) Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nat Rev Genet 12:499–510. doi:[10.1038/nrg3012](https://doi.org/10.1038/nrg3012)
- Davidson AD, Hewitt CL, Kashian DR (2015) Understanding acceptable level of risk: incorporating the economic cost of under-managing invasive species. PLoS ONE. doi:[10.1371/journal.pone.0141958](https://doi.org/10.1371/journal.pone.0141958)
- de Villemereuil P, Gaggiotti OE, Mouterde M, Till-Bottraud I (2015) Common garden experiments in the genomic era: new perspectives and opportunities. Heredity 116:249–254. doi:[10.1038/hdy.2015.93](https://doi.org/10.1038/hdy.2015.93)
- De Wit P, Palumbi SR (2013) Transcriptome-wide polymorphisms of red abalone (*Haliotis rufescens*) reveal patterns of gene flow and local adaptation. Mol Ecol 22:2884–2897. doi:[10.1111/mec.12081](https://doi.org/10.1111/mec.12081)
- De Wit P, Pespeni MH, Ladner JT, Barshis DJ, Seneca F, Jaris H, Therikildsen 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 Res 12:1058–1067. doi:[10.1111/1755-0998.12003](https://doi.org/10.1111/1755-0998.12003)
- De Wit P, Pespeni MH, Palumbi SR (2015) SNP genotyping and population genomics from expressed sequences -current advances and future possibilities. Mol Ecol. doi:[10.1111/mec.13165](https://doi.org/10.1111/mec.13165)
- Deagle BE, Jones FC, Absher DM, Kingsley DM, Reimchen TE (2013) Phylogeography and adaptation genetics of stickleback from the Haida Gwaii archipelago revealed using genome-wide single nucleotide polymorphism genotyping. Mol Ecol 22:1917–1932. doi:[10.1111/mec.12215](https://doi.org/10.1111/mec.12215)
- Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, Guernec G, Martin D, Merkel A, Knowles DG, Lagarde J, Veeravalli L, Ruan X, Ruan Y, Lassmann T, Carninci P, Brown JB, Lipovich L, Gonzalez JM, Thomas M, Davis CA, Shiekhattar R, Gingeras TR, Hubbard TJ, Notredame C, Harrow J, Guigó R (2012) The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res 22:1775–1789. doi:[10.1101/gr.132159.111](https://doi.org/10.1101/gr.132159.111)
- Díaz-Freije E, Gestal C, Castellanos-Martínez S, Morán P (2014) The role of DNA methylation on *Octopus vulgaris* development and their perspectives. Front Physiol. doi:[10.3389/fphys.2014.00062/full](https://doi.org/10.3389/fphys.2014.00062/full)
- Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol 17:431–449. doi:[10.1111/j.1365-294X.2007.03538.x](https://doi.org/10.1111/j.1365-294X.2007.03538.x)
- Dlugosch KM, Anderson SR, Braasch J, Cang FA, Gillette HD (2015) The devil is in the details: genetic variation in introduced populations and its contributions to invasion. Mol Ecol 24:2095–2111. doi:[10.1111/mec.13183](https://doi.org/10.1111/mec.13183)
- Driskell AC, Ané C, Burleigh JG, McMahon MM, O'Meara BC, Sanderson MJ (2004) Prospects for building the tree of life from large sequence databases. Science 306:1172–1174. doi:[10.1126/science.1102036](https://doi.org/10.1126/science.1102036)

- Duforet-Frebourg N, Bazin E, Blum MGB (2014) Genome scans for detecting footprints of local adaptation using a Bayesian factor model. *Mol Biol Evol* 31:2483–2495. doi:[10.1093/molbev/msu182](https://doi.org/10.1093/molbev/msu182)
- Edmonds CA, Lillie AS, Cavalli-Sforza LL (2004) Mutations arising in the wave front of an expanding population. *Proc Natl Acad Sci USA* 101:975–979. doi:[10.1073/pnas.0308064100](https://doi.org/10.1073/pnas.0308064100)
- Eichten SR, Swanson-Wagner RA, Schnable JC, Waters AJ, Hermanson PJ, Liu S, Yeh C-T, Jia Y, Gendler K, Freeling M (2011) Heritable epigenetic variation among maize inbreds. *PLoS Genet* 7:e1002372
- Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS ONE* 6:e19379. doi:[10.1371/journal.pone.0019379](https://doi.org/10.1371/journal.pone.0019379)
- Excoffier L, Ray N (2008) Surfing during population expansions promotes genetic revolutions and structuration. *Trends Ecol Evol* 23:347–351. doi:[10.1016/j.tree.2008.04.004](https://doi.org/10.1016/j.tree.2008.04.004)
- Fariello MI, Boitard S, Naya H, SanCristobal M, Servin B (2013) Detecting signatures of selection through haplotype differentiation among hierarchically structured populations. *Genetics* 193:929–941. doi:[10.1534/genetics.112.147231](https://doi.org/10.1534/genetics.112.147231)
- Feder ME, Walser J-C (2005) The biological limitations of transcriptomics in elucidating stress and stress responses. *J Evol Biol* 18:901–910. doi:[10.1111/j.1420-9101.2005.00921.x](https://doi.org/10.1111/j.1420-9101.2005.00921.x)
- 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. doi:[10.1242/jeb.02036](https://doi.org/10.1242/jeb.02036)
- Fierst JL (2015) Using linkage maps to correct and scaffold de novo genome assemblies: methods, challenges, and computational tools. *Front Genet* 6:1–8. doi:[10.3389/fgene.2015.00220](https://doi.org/10.3389/fgene.2015.00220)
- Fletcher LM, Forrest BM (2011) Induced spawning and culture techniques for the invasive ascidian *Didemnum vexillum* (Kott, 2002). *Aquat Invasions* 6:457–464. doi:[10.3391/ai.2011.6.4.11](https://doi.org/10.3391/ai.2011.6.4.11)
- Fofonoff PW, Ruiz GM, Steves B, Carlton JT (2003) In ships or on ships? Mechanisms of transfer and invasion for nonnative species to the coasts of North America. In: Ruiz GM & Carlton JT (eds) *Invasive species: Vectors and management strategies*. Island Press, Washington, pp 152–182
- Foll M, Gaggiotti O (2008) A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. *Genetics* 180:977–993. doi:[10.1534/genetics.108.092221](https://doi.org/10.1534/genetics.108.092221)
- François O, Martins H, Caye K, Schoville SD (2015) Controlling false discoveries in genome scans for selection. *Mol Ecol*. doi:[10.1111/mec.13513](https://doi.org/10.1111/mec.13513)
- Frankham R, Ballou JD, Briscoe DA (2009) *Introduction to conservation genetics*. Cambridge University Press, Cambridge
- Frankham R, Ballou JD, Eldridge MD, Lacy RC, Ralls K, Dudash MR, Fenster CB (2011) Predicting the probability of outbreeding depression. *Conserv Biol J Soc Conserv Biol* 25:465–475. doi:[10.1111/j.1523-1739.2011.01662.x](https://doi.org/10.1111/j.1523-1739.2011.01662.x)
- Franssen SU, Gu J, Bergmann N, Winters G, Klostermeier UC, Rosenstiel P, Bornberg-Bauer E, Reusch TBH (2011) Transcriptomic resilience to global warming in the seagrass *Zostera marina*, a marine foundation species. *Proc Natl Acad Sci USA* 108:19276–19281
- Fraser HB (2011) Genome-wide approaches to the study of adaptive gene expression evolution: systematic studies of evolutionary adaptations involving gene expression will allow many fundamental questions in evolutionary biology to be addressed. *BioEssays* 33:469–477. doi:[10.1002/bies.201000094](https://doi.org/10.1002/bies.201000094)
- Fraser HB (2013) Gene expression drives local adaptation in humans. *Genome Res* 23:1089–1096. doi:[10.1101/gr.152710.112](https://doi.org/10.1101/gr.152710.112)
- Fraser HB, Moses AM, Schadt EE (2010) Evidence for widespread adaptive evolution of gene expression in budding yeast. *Proc Natl Acad Sci USA* 107:2977–2982. doi:[10.1073/pnas.0912245107](https://doi.org/10.1073/pnas.0912245107)
- Futschik A, Schlotterer C (2010) The next generation of molecular markers from massively parallel sequencing of pooled DNA samples. *Genetics* 186:207–218. doi:[10.1534/genetics.110.114397](https://doi.org/10.1534/genetics.110.114397)
- Gautier M (2015) Genome-wide scan for adaptive divergence and association with population-specific covariates. *Genetics* 201:1555–1579. doi:[10.1534/genetics.115.181453](https://doi.org/10.1534/genetics.115.181453)
- Gautier M, Gharbi K, Cezard T, Foucaud J (2012) The effect of RAD allele dropout on the estimation of genetic variation within and between populations. *Mol Ecol*. doi:[10.1111/mec.12089](https://doi.org/10.1111/mec.12089)
- Gavery MR, Roberts SB (2010) DNA methylation patterns provide insight into epigenetic regulation in the Pacific oyster (*Crassostrea gigas*). *BMC Genom* 11:1
- Gayral P, Melo-Ferreira J, Glemin S, Bierne N, Carneiro M, Nabholz B, Lourenco JM, Alves PC, Ballenghien M, Faivre N, Belkhir K, Cahais V, Loire E, Bernard A, Galtier N (2013) Reference-free population genomics from next-generation transcriptome data and the vertebrate-invertebrate gap. *PLoS Genet*. doi:[10.1371/journal.pgen.1003457](https://doi.org/10.1371/journal.pgen.1003457)
- Geller JB, Walton ED, Grosholz ED, Ruiz GM (1997) Cryptic invasions of the crab *Carcinus* detected by molecular phylogeography. *Mol Ecol* 6:901–906. doi:[10.1046/j.1365-294X.1997.00256.x](https://doi.org/10.1046/j.1365-294X.1997.00256.x)
- Gerard K, Bierne N, Borsa P, Chenuil A, Feral JP (2008) Pleistocene separation of mitochondrial lineages of *Mytilus* spp. mussels from Northern and Southern Hemispheres and strong genetic differentiation among southern populations. *Mol Phylogenet Evol* 49:84–91. doi:[10.1016/j.ympev.2008.07.006](https://doi.org/10.1016/j.ympev.2008.07.006)
- Ghahooli S, Shiganova TA, Zhan A, Cristescu ME, Eghtesadi-Araghi P, MacIsaac HJ (2011) Multiple introductions and invasion pathways for the invasive ctenophore *Mnemiopsis leidyi* in Eurasia. *Biol Invas* 13:679–690. doi:[10.1007/s10530-010-9859-8](https://doi.org/10.1007/s10530-010-9859-8)
- Gleason LU, Burton RS (2015) RNA-seq reveals regional differences in transcriptome response to heat stress in the marine snail *Chlorostoma funebris*. *Mol Ecol* 24:610–627. doi:[10.1111/mec.13047](https://doi.org/10.1111/mec.13047)
- Gorokhova E, Lehtiniemi M, Viitasalo-Frosen S, Haddock SHD (2009) Molecular evidence for the occurrence of ctenophore *Mertensia ovum* in the northern Baltic Sea and implications for the status of the *Mnemiopsis leidyi* invasion. *Limnol Oceanogr* 54:2025–2033. doi:[10.4319/lo.2009.54.6.2025](https://doi.org/10.4319/lo.2009.54.6.2025)
- Gracey AY, Fraser EJ, Li W, Fang Y, Taylor RR, Rogers J, Brass A, Cossins AR (2004) Coping with cold: an integrative, multitissue analysis of the transcriptome of a poikilothermic vertebrate. *Proc Natl Acad Sci USA* 101:16970–16975. doi:[10.1073/pnas.0403627101](https://doi.org/10.1073/pnas.0403627101)
- Greco M, Chiappetta A, Bruno L, Bitonti MB (2011) In *Posidonia oceanica* cadmium induces changes in DNA methylation and chromatin patterning. *J Exp Bot*: err313
- Grosholz E (2002) Ecological and evolutionary consequences of coastal invasions. *Trends Ecol Evol* 17:22–27. doi:[10.1016/S0169-5347\(01\)02358-8](https://doi.org/10.1016/S0169-5347(01)02358-8)
- Grulois D, Leveque L, Viard F (2011) Mosaic genetic structure and sustainable establishment of the invasive kelp *Undaria pinnatifida* within a bay (Bay of St-Malo, Brittany). *Cah Biol Mar* 52:485–498
- Günther T, Coop G (2013) Robust identification of local adaptation from allele frequencies. *Genetics* 195:205–220. doi:[10.1534/genetics.113.152462](https://doi.org/10.1534/genetics.113.152462)
- Guo B, Li Z, Merilä J (2016) Population genomic evidence for adaptive differentiation in the Baltic Sea herring. *Mol Ecol*. doi:[10.1111/mec.13657](https://doi.org/10.1111/mec.13657)
- Gupta V, Bijo A, Kumar M, Reddy C, Jha B (2012) Detection of epigenetic variations in the protoplast-derived germlings of *Ulva*

- reticulata* using methylation sensitive amplification polymorphism (MSAP). *Mar Biotechnol* 14:692–700
- Hänfling B, Edwards F, Gherardi F (2011) Invasive alien Crustacea: dispersal, establishment, impact and control. *Biocontrol*. doi:10.1007/s10526-011-9380-8
- Harms L, Frickenhaus S, Schiffer M, Mark FC, Storch D, Held C, Pörtner H-O, Lucassen M (2014) Gene expression profiling in gills of the great spider crab *Hyas araneus* in response to ocean acidification and warming. *BMC Genom* 15:789. doi:10.1186/1471-2164-15-789
- Hastings A, Cuddington K, Davies KF, Dugaw CJ, Elmendorf S, Freestone A, Harrison S, Holland M, Lambrinos J, Malvadkar U, Melbourne BA, Moore K, Taylor C, Thomson D (2005) The spatial spread of invasions: new developments in theory and evidence. *Ecol Lett* 8:91–101. doi:10.1111/j.1461-0248.2004.00687.x
- Hauser L, Carvalho GR (2008) Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. *Fish Fish* 9:333–362. doi:10.1111/j.1467-2979.2008.00299.x
- He Y, Du Y, Li J, Liu P, Wang Q, Li Z (2015) Analysis of DNA methylation in different tissues of *Fenneropenaeus chinensis* from the wild population and Huanghai No. 1. *Acta Oceanologica Sinica* 34:175–180
- Hedrick PW (2011) Genetics of populations, Fourth edn. Jones and Bartlett, Sudbury
- Hendry AP (2016) Key questions on the role of phenotypic plasticity in eco-evolutionary dynamics. *J Hered* 107:25–41. doi:10.1093/jhered/esv060
- Herborg L-M, Weetman D, Van Oosterhout C, Hanfling B (2007) Genetic population structure and contemporary dispersal patterns of a recent European invader, the Chinese mitten crab, *Eriocheir sinensis*. *Mol Ecol* 16:231–242. doi:10.1111/j.1365-294X.2006.03133.x
- 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. doi:10.1111/mec.12150
- Hewitt CL (2003) Marine biosecurity issues in the world oceans: global activities and Australian directions. *Ocean Yearbook* 17(17):193–212
- Hewitt GM (2004) Genetic consequences of climatic oscillations in the quaternary. *Philos Trans R Soc Lond Ser B Biol Sci* 359:183–195. doi:10.1098/rstb.2003.1388
- Hewitt CL, Campbell ML (2007) Mechanisms for the prevention of marine bioinvasions for better biosecurity. *Mar Pollut Bull* 55:395–401. doi:10.1016/j.marpolbul.2007.01.005
- Hilbish TJ, Mullinax A, Dolven SI, Meyer A, Koehn RK, Rawson PD (2000) Origin of the antitropical distribution pattern in marine mussels (*Mytilus* spp.): routes and timing of transequatorial migration. *Mar Biol* 136:69–77
- Hill MP, Hoffmann AA, Umina PA, Cheng X, Miller AD (2016) Genetic analysis along an invasion pathway reveals endemic cryptic taxa, but a single species with little population structure in the introduced range. *Divers Distrib* 22:57–72. doi:10.1111/ddi.12385
- Hoban S, Kelley J, Lotterhos K, Antolin M, Bradburd G, Lowry D, Poss M, Reed L, Storfer A, Whitlock M (2016) Finding the genomic basis of local adaptation: pitfalls, practical solutions and future directions *American Naturalist*
- Hochachka P, Somero G (2002) Biochemical adaptation: mechanism and process in physiological evolution. Oxford University Press, Oxford
- Hoffmann AA, Willi Y (2008) Detecting genetic responses to environmental change. *Nat Rev Genet* 9:421–432
- Holderegger R, Kamm U, Gugerli F (2006) Adaptive vs. neutral genetic diversity: implications for landscape genetics. *Landscape Ecol* 21:797–807. doi:10.1007/s10980-005-5245-9
- Hollander J, Collyer ML, Adams DC, Johannesson K (2006) Phenotypic plasticity in two marine snails: constraints superseding life history. *J Evol Biol* 19:1861–1872. doi:10.1111/j.1420-9101.2006.01171.x
- Holt RD, Gomulkiewicz R (1997) how does immigration influence local adaptation? A reexamination of a familiar paradigm. *Am Nat* 149:563–572. doi:10.1086/286005
- Hui M, Liu Y, Song C, Li Y, Shi G, Cui Z (2014) Transcriptome changes in eriocheir sinensis megalopae after desalination provide insights into osmoregulation and stress adaptation in larvae. *PLoS ONE* 9:e114187. doi:10.1371/journal.pone.0114187
- Hutchings JA, Swain DP, Rowe S, Eddington JD, Puvanendran V, Brown JA (2007) Genetic variation in life-history reaction norms in a marine fish. *Proc R Soc Lond B Biol Sci* 274:1693–1699. doi:10.1098/rspb.2007.0263
- Johnson MS, Black R (1984) Pattern beneath the Chaos—the effect of recruitment on genetic patchiness in an intertidal limpet. *Evolution* 38:1371–1383
- Jones MR, Good JM (2015) Targeted capture in evolutionary and ecological genomics. *Mol Ecol* 25:185–202. doi:10.1111/mec.13304
- Jurka J, Kapitonov VV, Pavlicek A, Klonowski P, Kohany O, Walichewicz J (2005) Repbase update, a database of eukaryotic repetitive elements. *Cytogenet Genome Res* 110:462–467. doi:10.1159/000084979
- Karlsen BO, Klingan K, Emblem Å, Jørgensen TE, Jueterbock A, Furmanek T, Hoarau G, Johansen SD, Nordeide JT, Moun T (2013) Genomic divergence between the migratory and stationary ecotypes of Atlantic cod. *Mol Ecol* 22:5098–5111. doi:10.1111/mec.12454
- Kelley AL, de Rivera CE, Buckley BA (2011) Intraspecific variation in thermotolerance and morphology of the invasive European green crab, *Carcinus maenas*, on the west coast of North America. *J Exp Mar Biol Ecol* 409:70–78. doi:10.1016/j.jembe.2011.08.005
- Kilvitis H, Alvarez M, Foust C, Schrey A, Robertson M, Richards C (2014) Ecological epigenetics. In: Landry CR, Aubin-Horth N (eds) *Ecological genomics*. Springer, Berlin, pp 191–210
- Kirk H, Dorn S, Mazzi D (2013) Molecular genetics and genomics generate new insights into invertebrate pest invasions. *Evol Appl* 6:842–856. doi:10.1111/eva.12071
- Klopfstein S, Currat M, Excoffier L (2006) The fate of mutations surfing on the wave of a range expansion. *Mol Biol Evol* 23:482–490. doi:10.1093/molbev/msj057
- Knott KE, Wray Ga (2000) Controversy and consensus in asteroid systematics: new insights to ordinal and familial relationships. *Am Zool* 40:382–392
- Koskinen P, Törönen P, Nokso-Koivisto J, Holm L (2015) PANZER: high-throughput functional annotation of uncharacterized proteins in an error-prone environment. *Bioinformatics* 31:1544–1552. doi:10.1093/bioinformatics/btu851
- Laine V, Gossmann T, Schachtschneider K, Garroway C, Madsen O, Verhoeven K, de Jager V, Megens H, Warren W, Minx P (2015) Evolutionary signals of selection on cognition from the great tit genome and methylome. *Nat Commun*
- Lal MM, Southgate PC, Jerry DR, Zenger KR (2016) Fishing for divergence in a sea of connectivity: the utility of ddRADseq genotyping in a marine invertebrate, the black-lip pearl oyster *Pinctada margaritifera*. *Mar Genom* 25:57–68. doi:10.1016/j.margen.2015.10.010
- Landy JA, Travis J (2015) Shape variation in the least killifish: ecological associations of phenotypic variation and the effects of a common garden. *Ecol Evol* 5:5616–5631. doi:10.1002/ece3.1780
- Larsen PF, Nielsen EE, Williams TD, Hemmer-Hansen J, Chipman JK, Krühøffer M, Grønkjær P, George SG, Dyrskjøt L, Loeschcke V (2007) Adaptive differences in gene expression

- in European flounder (*Platichthys flesus*). *Mol Ecol* 16:4674–4683. doi:10.1111/j.1365-294X.2007.03530.x
- Larsen PF, Schulte PM, Nielsen EE (2011) Gene expression analysis for the identification of selection and local adaptation in fishes. *J Fish Biol* 78:1–22. doi:10.1111/j.1095-8649.2010.02834.x
- Lee CE (2002) Evolutionary genetics of invasive species. *Trends Ecol Evol* 17:386–391. doi:10.1016/s0169-5347(02)02554-5
- Lenz PH, Roncalli V, Hassett RP, Wu L-S, Cieslak MC, Hartline DK, Christie AE (2014) De Novo assembly of a transcriptome for *Calanus finmarchicus* (Crustacea, Copepoda)? The dominant zooplankton of the North Atlantic Ocean. *PLoS ONE* 9:e88589. doi:10.1371/journal.pone.0088589
- Lewis PN, Hewitt CL, Riddle M, McMinn A (2003) Marine introductions in the Southern Ocean: an unrecognised hazard to biodiversity. *Mar Pollut Bull* 46:213–223. doi:10.1016/s0025-326x(02)00364-8
- Li S, Qiao K, Shan T, Pang S, Hou H (2013a) Genetic diversity and relationships of the brown alga *Undaria pinnatifida* cultivated along the Dalian Coast as revealed by amplified fragment length polymorphism markers. *J Appl Phycol* 25:1255–1263. doi:10.1007/s10811-012-9922-1
- Li X, Cui Z, Liu Y, Song C, Shi G (2013b) Transcriptome analysis and discovery of genes involved in immune pathways from hepatopancreas of microbial challenged mitten crab *Eriocheir sinensis*. *PLoS ONE*. doi:10.1371/journal.pone.0068233
- Li E, Wang S, Li C, Wang X, Chen K, Chen L (2014) Transcriptome sequencing revealed the genes and pathways involved in salinity stress of Chinese mitten crab, *Eriocheir sinensis*. *Physiol Genom* 46:177–190. doi:10.1152/physiolgenomics.00191.2013
- Li Y, Huang X, Guan Y, Shi Y, Zhang H, He M (2015) DNA methylation is associated with expression level changes of galectin gene in mantle wound healing process of pearl oyster, *Pinctada fucata*. *Fish Shellfish Immunol* 45:912–918
- Liebl AL, Schrey AW, Richards CL, Martin LB (2013) Patterns of DNA methylation throughout a range expansion of an introduced songbird. *Integr Comp Biol* 53:351–358. doi:10.1093/icb/ict007
- Limborg MT, Helyar SJ, De Bruyn M, Taylor MI, Nielsen EE, Ogden ROB, Carvalho GR, Consortium FPT, 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. doi:10.1111/j.1365-294X.2012.05639.x
- Liu Y, Zhou J, White KP (2014) RNA-seq differential expression studies: more sequence or more replication? *Bioinformatics* 30:301–304. doi:10.1093/bioinformatics/btt688
- Lockwood BL, Somero GN (2011) Transcriptomic responses to salinity stress in invasive and native blue mussels (genus *Mytilus*). *Mol Ecol* 20:517–529. doi:10.1111/j.1365-294X.2010.04973.x
- 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. *The Journal of experimental biology* 213:3548–3558. doi:10.1242/jeb.046094
- Lombaert E, Estoup A, Facon B, Joubard B, Grégoire JC, Jannin A, Blin A, Guillemaud T (2014) Rapid increase in dispersal during range expansion in the invasive ladybird *Harmonia axyridis*. *J Evol Biol* 27:508–517
- Lotterhos KE, Schaal SM (2014) Genome scans for the contemporary response to selection in quantitative traits. *Mol Ecol* 23:4435–4437. doi:10.1111/mec.12853
- Lotterhos KE, Whitlock MC (2014) Evaluation of demographic history and neutral parameterization on the performance of  $F_{ST}$  outlier tests. *Mol Ecol* 23:2178–2192. doi:10.1111/mec.12725
- Lu B, Zeng Z, Shi T (2013) Comparative study of de novo assembly and genome-guided assembly strategies for transcriptome reconstruction based on RNA-Seq. *Sci China Life Sci* 56:143–155. doi:10.1007/s11427-013-4442-z
- Lv J, Liu P, Wang Y, Gao B, Chen P, Li J (2013) Transcriptome analysis of portunus trituberculatus in response to salinity stress provides insights into the molecular basis of osmoregulation. *PLoS ONE* 8:e82155. doi:10.1371/journal.pone.0082155
- Mamanova L, Coffey AJ, Scott CE, Kozarewa I, Turner EH, Kumar A, Howard E, Shendure J, Turner DJ (2010) Target-enrichment strategies for next-generation sequencing. *Nat Methods* 7:111–118. doi:10.1038/nmeth.1419
- Massicotte R, Whitelaw E, Angers B (2011) DNA methylation: a source of random variation in natural populations. *Epigenetics* 6:421–427
- Matsuoka N, Hatanaka T (1998) Genetic differentiation among local Japanese populations of the starfish *Asterias amurensis* inferred from allozyme variation. *Genes Genetic Syst* 73:59–64. doi:10.1266/ggs.73.59
- McDonald JH, Koehn RK (1988) The mussels *Mytilus galloprovincialis* and *M. trossulus* on the Pacific coast of North America. *Mar Biol* 99:111–118. doi:10.1007/bf00644984
- McDonald JH, Seed R, Koehn RK (1991) Allozymes and morphometric characters of three species of *Mytilus* in the Northern and Southern Hemispheres. *Mar Biol* 111:323–333. doi:10.1007/bf01319403
- Mendizabal I, Keller TE, Zeng J, Yi SV (2014) Epigenetics and evolution. *Integr Comp Biol* 54:31–42. doi:10.1093/icb/ucu040
- Messer PW, Petrov DA (2013) Population genomics of rapid adaptation by soft selective sweeps. *Trends Ecol Evol* 28:659–669. doi:10.1016/j.tree.2013.08.003
- Metzger DC, Schulte PM (2016) Epigenomics in marine fishes. *Mar Genom*. doi:10.1016/j.margen.2016.01.004
- Metzger BPH, Duveau F, Yuan DC, Tryban S, Yang B, Wittkopp PJ (2016) Contrasting frequencies and effects of cis- and trans-regulatory mutations affecting gene expression. *Mol Biol Evol*. doi:10.1093/molbev/msw011
- Meunier I, Valero M, Destombe C, Gode C, Desmarais E, Bonhomme F, Stam WT, Olsen JL (2002) Polymerase chain reaction-single strand conformation polymorphism analyses of nuclear and chloroplast DNA provide evidence for recombination, multiple introductions and nascent speciation in the *Caulerpa taxifolia* complex. *Mol Ecol* 11:2317–2325. doi:10.1046/j.1365-294X.2002.01627.x
- Miga KH (2015) Completing the human genome: the progress and challenge of satellite DNA assembly. *Chromosome Res* 23:421–426. doi:10.1007/s10577-015-9488-2
- Milano I, Babbucci M, Cariani A, Atanassova M, Bekkevold D, Carvalho GR, Espiñeira M, Fiorentino F, Garofalo G, Geffen AJ, Hansen JH, Helyar SJ, Nielsen EE, Ogden R, Patarnello T, Stagoni M, FishPopTrace C, Tinti F, Bargelloni L (2014) Outlier SNP markers reveal fine-scale genetic structuring across European hake populations (*Merluccius merluccius*). *Mol Ecol* 23:118–135. doi:10.1111/mec.12568
- Miller AD, Skoracka A, Navia D, de Mendonca RS, Szydlow W, Schultz MB, Smith CM, Truol G, Hoffmann AA (2013) Phylogenetic analyses reveal extensive cryptic speciation and host specialization in an economically important mite taxon. *Mol Phylogenet Evol* 66:928–940. doi:10.1016/j.ympev.2012.11.021
- Möller LF, Jonsson P, Reuch T, Dupont S (2014) Impact of the rate of change on adaptation: response of *Mnemiopsis leidyi* to low salinity through multiple generations. Centre for Marine Evolutionary Biology 11th assembly
- 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. doi:10.1890/070064

- Moreira R, Pereiro P, Canchaya C, Posada D, Figueras A, Novoa B (2015) RNA-Seq in *Mytilus galloprovincialis*: comparative transcriptomics and expression profiles among different tissues. *BMC Genom* 16:1–18. doi:10.1186/s12864-015-1817-5
- Moreton J, Izquierdo A, Emes RD (2016) Assembly, assessment, and availability of de novo generated eukaryotic transcriptomes. *Front Genetics* 6:361. doi:10.3389/fgene.2015.00361
- Morgan HD, Santos F, Green K, Dean W, Reik W (2005) Epigenetic reprogramming in mammals. *Hum Mol Genet* 14:R47–R58. doi:10.1093/hmg/ddi114
- Morozova O, Marra MA (2008) Applications of next-generation sequencing technologies in functional genomics. *Genomics* 92:255–264. doi:10.1016/j.ygeno.2008.07.001
- Muraoka D, Saitoh K (2005) Identification of *Undaria pinnatifida* and *Undaria undarioides* Laminariales, Phaeophyceae using mitochondrial 23S ribosomal DNA sequences. *Fish Sci* 71:1365–1369. doi:10.1111/j.1444-2906.2005.01103.x
- Murgarella M, Puiu D, Novoa B, Figueras A, Posada D, Canchaya C (2016) A first insight into the genome of the filter-feeder mussel *Mytilus galloprovincialis*. *PLoS ONE* 11:e0151561. doi:10.1371/journal.pone.0151561
- Murphy N, Evans B (1998) Genetic origin of Australian populations of *Asterias amurensis*. In: Goggin L (ed) Proceedings of a meeting on the biology and management of the introduced seastar *Asterias amurensis* in Australian waters. CSIRO Division of Marine Research, Hobart, pp 22–25
- Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. *Evolution* 29:1–10. doi:10.2307/2407137
- Novák P, Neumann P, Macas J (2010) Graph-based clustering and characterization of repetitive sequences in next-generation sequencing data. *BMC Bioinform* 11:378. doi:10.1186/1471-2105-11-378
- Ojaveer H, Galil BS, Campbell ML, Carlton JT, Canning-Clode J, Cook EJ, Davidson AD, Hewitt CL, Jelmert A, Marchini A, McKenzie CH, Minchin D, Occhipinti-Ambrogi A, Olenin S, Ruiz G (2015) Classification of non-indigenous species based on their impacts: considerations for application in marine management. *PLoS Biol*. doi:10.1371/journal.pbio.1002130
- Olsen JL, Valero M, Meusnier I, Boele-Bos S, Stam WT (1998) Mediterranean *Caulerpa taxifolia* and *C. mexicana* (Chlorophyta) are not conspecific. *J Phycol* 34:850–856
- Pang K, Martindale MQ (2008) Developmental expression of homeobox genes in the ctenophore *Mnemiopsis leidyi*. *Dev Genes Evol* 218:307–319. doi:10.1007/s00427-008-0222-3
- Pavy N, Gagnon F, Deschênes A, Boyle B, Beaulieu J, Bousquet J (2015) Development of highly reliable in silico SNP resource and genotyping assay from exome capture and sequencing: an example from black spruce (*Picea mariana*). *Mol Ecol Resour*. doi:10.1111/1755-0998.12468
- Pearse DE, Crandall KA (2004) Beyond F(ST): analysis of population genetic data for conservation. *Conserv Genet* 5:585–602. doi:10.1007/s10592-003-1863-4
- Pérez J, Nirchio M, Alfonsi C, Muñoz C (2006) The biology of invasions: the genetic adaptation paradox. *Biol Invas* 8:1115–1121. doi:10.1007/s10530-005-8281-0
- Pespeni MH, Sanford E, Gaylord B, Hill TM, Hosfelt JD, Jaris HK, LaVigne M, Lenz EA, Russell AD, Young MK, Palumbi SR (2013) Evolutionary change during experimental ocean acidification. *Proc Natl Acad Sci USA* 110:6937–6942. doi:10.1073/pnas.1220673110
- Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE (2012) Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS ONE* 7:e37135. doi:10.1371/journal.pone.0037135
- Phillips BL, Brown GP, Webb JK, Shine R (2006) Invasion and the evolution of speed in toads. *Nature* 439: 803–803. [http://www.nature.com/nature/journal/v439/n7078/suppinfo/439803a\\_S1.html](http://www.nature.com/nature/journal/v439/n7078/suppinfo/439803a_S1.html)
- Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecol Econ* 52:273–288. doi:10.1016/j.ecolecon.2004.10.002
- Platt Ii RN, Blanco-Berdugo L, Ray DA (2016) Accurate transposable element annotation is vital when analyzing new genome assemblies. *Genome Biol Evol Adv Access* 8:403–410. doi:10.1093/gbe/evw009
- Poelchau MF, Coates BS, Childers CP, Pérez de León AA, Evans JD, Hackett K, Shoemaker D (2016) Agricultural applications of insect ecological genomics. *Curr Opin Insect Sci* 13:61–69
- 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* 108:15288–15293. doi:10.1073/pnas.1100473108
- Pritchard JK, Di Rienzo A (2010) Adaptation—not by sweeps alone. *Nat Rev Genet* 11:665–667
- Puritz JB, Hollenbeck CM, Gold JR (2014a) dDocent: a RADseq, variant-calling pipeline designed for population genomics of non-model organisms. *Peer J* 2:e431. doi:10.7717/peerj.431
- Puritz JB, Matz MV, Toonen RJ, Weber JN, Bolnick DI, Bird CE (2014b) Demystifying the RAD fad. *Mol Ecol* 23:5937–5942. doi:10.1111/mec.12965
- Quek XC, Thomson DW, Maag JLV, Bartonicek N, Signal B, Clark MB, Gloss BS, Dinger ME (2015) lncRNADB v2.0: expanding the reference database for functional long noncoding RNAs. *Nucleic Acids Res* 43:D168–D173. doi:10.1093/nar/gku988
- Rakyan VK, Chong S, Champ ME, Cuthbert PC, Morgan HD, Luu KV, Whitelaw E (2003) Transgenerational inheritance of epigenetic states at the murine *AxinFu* allele occurs after maternal and paternal transmission. *Proc Natl Acad Sci* 100:2538–2543
- Ranjan A, Townsley BT, Ichihashi Y, Sinha NR, Chitwood DH (2015) An intracellular transcriptomic atlas of the giant coenocyte *Caulerpa taxifolia*. *PLoS Genet*. doi:10.1371/journal.pgen.1004900
- Rapp RA, Wendel JF (2005) Epigenetics and plant evolution. *New Phytol* 168:81–91
- Rellstab C, Gugerli F, Eckert AJ, Hancock AM, Holderegger R (2015) A practical guide to environmental association analysis in landscape genomics. *Mol Ecol* 24:4348–4370. doi:10.1111/mec.13322
- Reusch TBH, Bolte S, Sparwel M, Moss AG, Javidpour J (2010) Microsatellites reveal origin and genetic diversity of Eurasian invasions by one of the world's most notorious marine invader, *Mnemiopsis leidyi* (Ctenophora). *Mol Ecol* 19:2690–2699. doi:10.1111/j.1365-294X.2010.04701.x
- Richards CL, Verhoeven KJ, Bosserdorf O (2012) Evolutionary significance of epigenetic variation. Springer, Berlin
- Richardson MF, Sherman CDH (2015) De novo assembly and characterization of the invasive Northern Pacific Seastar transcriptome. *PLoS ONE* 10:1–18. doi:10.1371/journal.pone.0142003
- Richardson MF, Sherman CDH, Lee RS, Bott NJ, Hirst AJ (2016) Multiple dispersal vectors drive range expansion in an invasive marine species. *Mol Ecol*. doi:10.1111/mec.13817
- Rideout W, Coetzee GA, Olumi AF, Jones PA (1990) 5-Methylcytosine as an endogenous mutagen in the human LDL receptor and p53 genes. *Science* 249:1288–1290
- Riesgo A, Andrade SCS, Sharma PP, Novo M, Pérez-Porro AR, Vahtera V, González VL, Kawauchi GY, Giribet G (2012) Comparative description of ten transcriptomes of newly sequenced invertebrates and efficiency estimation of genomic sampling in non-model taxa. *Front Zool* 9:33. doi:10.1186/1742-9994-9-33

- Rilov G, Crooks J (2009) biological invasions in marine ecosystems: ecology conservation and management perspectives. Springer, Heidelberg
- Rius M, Bourne S, Hornsby HG, Chapman MA (2015) Applications of next-generation sequencing to the study of biological invasions. *Curr Zool* 61:488
- Rivière G (2014) Epigenetic features in the oyster *Crassostrea gigas* suggestive of functionally relevant promoter DNA methylation in invertebrates. *Front Physiol* 5:129. doi:10.3389/fphys.2014.00129
- Rollins L, Richardson MF, Shine R (2015) A genetic perspective on rapid evolution in cane toads (*Rhinella marina*). *Mol Ecol* 24:2264–2276
- Roman J (2006) Diluting the founder effect: cryptic invasions expand a marine invader's range. *Proc R Soc B Biol Sci* 273:2453–2459. doi:10.1098/rspb.2006.3597
- Roman J, Darling JA (2007) Paradox lost: genetic diversity and the success of aquatic invasions. *Trends Ecol Evol* 22:454–464. doi:10.1016/j.tree.2007.07.002
- Roman JOE, Palumbi SR (2004) A global invader at home: population structure of the green crab, *Carcinus maenas*, in Europe. *Mol Ecol* 13:2891–2898. doi:10.1111/j.1365-294X.2004.02255.x
- Romero IG, Ruvinsky I, Gilad Y (2012) Comparative studies of gene expression and the evolution of gene regulation. *Nat Rev Genet* 13:505–516
- Rosani U, Domeneghetti S, Pallavicini A, Venier P (2014) Target capture and massive sequencing of genes transcribed in *Mytilus galloprovincialis*. *BioMed Research International* 2014:9. doi:10.1155/2014/538549
- Ruiz GM, Carlton JT, Grosholz ED, Hines AH (1997) Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent, and consequences. *Am Zool* 37:621–632
- Ryan JF, Pang K, Schnitzler CE, Anh-Dao N, Moreland RT, Simmons DK, Koch BJ, Francis WR, Havlak P, Smith SA, Putnam NH, Haddock SHD, Dunn CW, Wolfsberg TG, Mullikin JC, Martindale MQ, Baxeavanis AD, Progra NCS (2013) The genome of the ctenophore *Mnemiopsis leidyi* and its implications for cell type evolution. *Science*. doi:10.1126/science.1242592
- Sabeti PC, Varilly P, Fry B, Lohmueller J, Hostetter E, Cotsapas C, Xie X, Byrne EH, McCarroll SA, Gaudet R, Schaffner SF, Lander ES (2007) Genome-wide detection and characterization of positive selection in human populations. *Nature* 449:913–918. [http://www.nature.com/nature/journal/v449/n7164/supinfo/nature06250\\_S1.html](http://www.nature.com/nature/journal/v449/n7164/supinfo/nature06250_S1.html)
- Samorodnitsky E, Datta J, Jewell BM, Hagopian R, Miya J, Wing MR, Damodaran S, Lippus JM, Reeser JW, Bhatt D, Timmers CD, Roychowdhury S (2015) Comparison of custom capture for targeted next-generation DNA sequencing. *J Mol Diagn* 17:64–75. doi:10.1016/j.jmoldx.2014.09.009
- Sanford E, Kelly MW (2011) Local adaptation in marine invertebrates. *Ann Rev Mar Sci* 3:509–535. doi:10.1146/annurev-marine-120709-142756
- Sanford E, Roth MS, Johns GC, Wares JP, Somero GN (2003) Local selection and latitudinal variation in a marine predator-prey interaction. *Science* 300:1135–1137. doi:10.1126/science.1083437
- Sax DF, Stachowicz JJ, Brown JH, Bruno JF, Dawson MN, Gaines SD, Grosberg RK, Hasting S A, Holt RD, Mayfield MM, O'Connor MI, Rice WR (2007) Ecological and evolutionary insights from species invasions. *Trends Ecol Evol* 22:465–471. doi:10.1016/j.tree.2007.06.009
- Schlamp F, van der Made J, Stambler R, Chesebrough L, Boyko AR, Messer PW (2016) Evaluating the performance of selection scans to detect selective sweeps in domestic dogs. *Mol Ecol* 25:342–356. doi:10.1111/mec.13485
- Schlötterer C, Tobler R, Kofler R, Nolte V (2014) Sequencing pools of individuals—mining genome-wide polymorphism data without big funding. *Nat Rev Genet* 15:749–763. doi:10.1038/nrg3803
- Schnitzler CE, Pang K, Powers ML, Reitzel AM, Ryan JF, Simmons D, Tada T, Park M, Gupta J, Brooks SY, Blakesley RW, Yokoyama S, Haddock SHD, Martindale MQ, Baxeavanis AD (2012) Genomic organization, evolution, and expression of photoprotein and opsin genes in *Mnemiopsis leidyi*: a new view of ctenophore photocytes. *BMC Biol*. doi:10.1186/1741-7007-10-107
- Schrey AW, Coon CA, Grispo MT, Awad M, Imboma T, McCoy ED, Mushinsky HR, Richards CL, Martin LB (2012) Epigenetic variation may compensate for decreased genetic variation with introductions: a case study using house sparrows (*Passer domesticus*) on two continents. *Genet Res Int*
- Schweyen H, Rozenberg A, Leese F (2014) Detection and removal of PCR duplicates in population genomic ddRAD studies by addition of a degenerate base region (DBR) in sequencing adapters. *Biol Bull* 227:146–160
- Scientists GCo, The Global Invertebrate Genomics Alliance (GIGA) (2014) Developing community resources to study diverse invertebrate genomes. *J Hered* 105:1–18. doi:10.1093/jhered/est084
- Scott GR, Johnston IA (2012) Temperature during embryonic development has persistent effects on thermal acclimation capacity in zebrafish. *Proc Natl Acad Sci* 109:14247–14252. doi:10.1073/pnas.1205012109
- Sexton JP, Hangartner SB, Hoffmann AA (2014) Genetic isolation by environment or distance: which pattern of gene flow is most common? *Evolution* 68:1–15. doi:10.1111/evo.12258
- Shan T, Pang S, Li J, Li X, Su L (2015a) Construction of a high-density genetic map and mapping of a sex-linked locus for the brown alga *Undaria pinnatifida* (Phaeophyceae) based on large scale marker development by specific length amplified fragment (SLAF) sequencing. *BMC Genom*. doi:10.1186/s12864-015-2184-y
- Shan TF, Pang SJ, Li J, Li X (2015b) De novo transcriptome analysis of the gametophyte of *Undaria pinnatifida* (Phaeophyceae). *J Appl Phycol* 27:1011–1019. doi:10.1007/s10811-014-0393-4
- Sherman CDH, Ayre DJ (2008) Fine-scale adaptation in a clonal sea anemone. *Evolution* 62:1373–1380. doi:10.1111/j.1558-5646.2008.00375.x
- Sherman CDH, Ab Rahim ES, Olsson M, Careau V (2015) The more pieces, the better the puzzle: sperm concentration increases gametic compatibility. *Ecol Evol* 5:4354–4364. doi:10.1002/ece3.1684
- Shiganova TA (1998) Invasion of the Black Sea by the ctenophore *Mnemiopsis leidyi* and recent changes in pelagic community structure. *Fish Oceanogr* 7:305–310. doi:10.1046/j.1365-2419.1998.00080.x
- Shine R, Brown GP, Phillips BL (2011) An evolutionary process that assembles phenotypes through space rather than through time. *Proc Natl Acad Sci*. doi:10.1073/pnas.1018989108
- Simmen MW, Leitgeb S, Charlton J, Jones SJ, Harris BR, Clark VH, Bird A (1999) Nonmethylated transposable elements and methylated genes in a chordate genome. *Science* 283:1164–1167
- Song L, Bian C, Luo Y, Wang L, You X, Li J, Qiu Y, Ma X, Zhu Z, Ma L, Wang Z, Lei Y, Qiang J, Li H, Yu J, Wong A, Xu J, Shi Q, Xu P (2016) Draft genome of the Chinese mitten crab *Eriocheir sinensis*. *Giga Sci* 5:5. doi:10.1186/s13742-016-0112-y
- Soria-Carrasco V, Gompert Z, Aa Comeault, Farkas TE, Parchman TL, Johnston JS, Buerkle CA, Feder JL, Bast J, Schwander T, Egan SP, Crespi BJ, Nosil P (2014) Stick insect genomes reveal natural selection's role in parallel speciation. *Science* 344:738–742. doi:10.1126/science.1252136
- Stephan W (2016) Signatures of positive selection: from selective sweeps at individual loci to subtle allele frequency changes in polygenic adaptation. *Mol Ecol* 25:79–88. doi:10.1111/mec.13288
- Stillman JH, Tagmount A (2009) Seasonal and latitudinal acclimatization of cardiac transcriptome responses to thermal stress in

- porcelain crabs, *Petrolisthes cinctipes*. *Mol Ecol* 18:4206–4226. doi:10.1111/j.1365-294X.2009.04354.x
- 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
- Sui L, Zhang F, Wang X, Bossier P, Sorgeloos P, Hänfling B (2009) Genetic diversity and population structure of the Chinese mitten crab *Eriocheir sinensis* in its native range. *Mar Biol* 156:1573–1583. doi:10.1007/s00227-009-1193-2
- Sulonen A-M, Ellonen P, Almusa H, Lepistö M, Eldfors S, Hannula S, Miettinen T, Tynismaa H, Salo P, Heckman C, Joensuu H, Raivio T, Suomalainen A, Saarela J (2011) Comparison of solution-based exome capture methods for next generation sequencing. *Genome Biol* 12:R94. doi:10.1186/gb-2011-12-9-r94
- Sun Y, Hou R, Fu X, Sun C, Wang S, Wang C, Li N, Zhang L, Bao Z (2014) Genome-wide analysis of DNA methylation in five tissues of zhikong scallop, *Chlamys farreri*. *PLoS one* 9:e86232
- Sun L, Liu H, Zhang L, Meng J (2015) IncRScan-SVM: a tool for predicting long non-coding RNAs using support vector machine. *PLoS ONE* 10:e0139654. doi:10.1371/journal.pone.0139654
- Tajima F (1989a) The effect of change in population size on DNA polymorphism. *Genetics* 123:597–601
- Tajima F (1989b) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123:585–595
- Tajima F (1996) The amount of DNA polymorphism maintained in a finite population when the neutral mutation rate varies among sites. *Genetics* 143:1457–1465
- Tenaillon MI, U'Ren J, Tenaillon O, Gaut BS (2004) Selection versus demography: a multilocus investigation of the domestication process in maize. *Mol Biol Evol* 21:1214–1225. doi:10.1093/molbev/msh102
- Tepolt CK (2015) Adaptation in marine invasion: a genetic perspective. *Biol Invas* 17:887–903. doi:10.1007/s10530-014-0825-8
- Tepolt CK, Palumbi SR (2014) Genetic correlates of local adaptation in the globally invasive European green crab, *Carcinus maenas*. *Integr Comp Biol* 54:E207–E207
- Tepolt CK, Palumbi SR (2015) Transcriptome sequencing reveals both neutral and adaptive genome dynamics in a marine invader. *Mol Ecol* 24:4145–4158. doi:10.1111/mec.13294
- Tepolt CK, Somero GN (2013) Cardiac thermal tolerance and acclimatory plasticity in diverse populations of the invasive green crab, *Carcinus maenas*. *Integr Comp Biol* 53:E213–E213
- Tepolt CK, Darling JA, Bagley MJ, Geller JB, Blum MJ, Grosholz ED (2009) European green crabs (*Carcinus maenas*) in the northeastern Pacific: genetic evidence for high population connectivity and current-mediated expansion from a single introduced source population. *Divers Distrib* 15:997–1009. doi:10.1111/j.1472-4642.2009.00605.x
- Tian M, Li Y, Jing J, Mu C, Du H, Dou J, Mao J, Li X, Jiao W, Wang Y, Hu X, Wang S, Wang R, Bao Z (2015) Construction of a high-density genetic map and quantitative trait locus mapping in the sea cucumber *Apostichopus japonicus*. *Sci Rep* 5:14852. doi:10.1038/srep14852
- Tiffin P, Ross-Ibarra J (2014) Advances and limits of using population genetics to understand local adaptation. *Trends Ecol Evol* 29:673–680. doi:10.1016/j.tree.2014.10.004
- Tin MMY, Rheindt FE, Cros E, Mikheyev AS (2015) Degenerate adaptor sequences for detecting PCR duplicates in reduced representation sequencing data improve genotype calling accuracy. *Mol Ecol Resour* 15:329–336. doi:10.1111/1755-0998.12314
- Todgham AE, Hofmann GE (2009) Transcriptomic response of sea urchin larvae *Strongylocentrotus purpuratus* to CO<sub>2</sub>-driven seawater acidification. *J Exp Biol* 212:2579–2594. doi:10.1242/jeb.032540
- Toro JE (1998) PCR-based nuclear and mtDNA markers and shell morphology as an approach to study the taxonomic status of the Chilean blue mussel, *Mytilus chilensis* (Bivalvia). *Aquat Living Resour* 11:347–353. doi:10.1016/S0990-7440(98)80006-5
- Uliano-Silva M, Americo JA, Brindeiro R, Dondero F, Prosdociomi F, Rebelo MDF (2014) Gene discovery through transcriptome sequencing for the invasive mussel *Limnoperna fortunei*. *PLoS ONE* 9:e102973. doi:10.1371/journal.pone.0102973
- Urban MC, Phillips BL, Skelly DK, Shine R (2008) A toad more traveled: the heterogeneous invasion dynamics of cane toads in Australia. *Am Nat* 171:E134–E148. doi:10.1086/527494
- Uwai S, Nelson W, Neill K, Boo SM, Kawai H (2005) Genetic diversity of *Undaria pinnatifida* in Asia and New Zealand deduced from mitochondrial genes. *Phycologia* 44:52–53
- Uwai S, Nelson W, Neill K, Wang WD, Aguilar-Rosas LE, Boo SM, Kitayama T, Kawa H (2006a) Genetic diversity in *Undaria pinnatifida* (Laminariales, Phaeophyceae) deduced from mitochondrial genes origins and succession of introduced populations. *Phycologia* 45:687–695. doi:10.2216/05-66.1
- Uwai S, Yotsukura N, Serisawa Y, Muraoka D, Hiraoka M, Kogame K (2006b) Intraspecific genetic diversity of *Undaria pinnatifida* in Japan, based on the Mitochondrial *cox3* Gene and the ITS1 of nrDNA. *Hydrobiologia* 553:345–356. doi:10.1007/s10750-005-0883-0
- Uwai S, Emura N, Morita T, Kurashima A, Kawai H (2009) The genetic structure of *Undaria* species around Japan. *Phycologia* 48:135
- Van Petegem KHP, Pétillon J, Renault D, Wybouw N, Van Leeuwen T, Stoks R, Bonte D (2015) Empirically simulated spatial sorting points at fast epigenetic changes in dispersal behaviour. *Evol Ecol* 29:299–310
- Vandepitte K, de Meyer T, Helsen K, van Acker K, Roldán-Ruiz I, Mergeay J, Honnay O (2014) Rapid genetic adaptation precedes the spread of an exotic plant species. *Mol Ecol* 23:2157–2164. doi:10.1111/mec.12683
- Vanderhoeven S, Brown CS, Tepolt CK, Tsutsui ND, Vanparys V, Atkinson S, Mahy G, Monty A (2010) Linking concepts in the ecology and evolution of invasive plants: network analysis shows what has been most studied and identifies knowledge gaps. *Evol Appl* 3:193–202. doi:10.1111/j.1752-4571.2009.00116.x
- Vatsiou AI, Bazin E, Gaggiotti OE (2015) Detection of selective sweeps in structured populations: a comparison of recent methods. *Mol Ecol*. doi:10.1111/mec.13360
- Verbruggen B, Bickley LK, Santos EM, Tyler CR, Stentiford GD, Bateman KS, van Aerle R (2015) De novo assembly of the *Carcinus maenas* transcriptome and characterization of innate immune system pathways. *BMC Genom* 16:1–17. doi:10.1186/s12864-015-1667-1
- Verhoeven KJF, vonHoldt BM, Sork VL (2016) Epigenetics in ecology and evolution: what we know and what we need to know. *Mol Ecol*. doi:10.1111/mec.13617
- Voisin M, Engel C, Viard F (2005) Unravelling the mechanisms behind introduction through genetic approaches: *Undaria pinnatifida* (Alariaceae) as a model species. *Phycologia* 44:106–107
- Waddington CH (1953) Genetic assimilation of an acquired character. *Evolution* 7:118–126
- Waples RS (1998) Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *J Hered* 89:438–450. doi:10.1093/jhered/89.5.438
- Waples RS (2016) How plasticity and evolution work in the real World. *J Hered* 107:1–2. doi:10.1093/jhered/esv093
- Ward RD, Andrew J (1995) Population genetics of the northern Pacific seastar *Asterias amurensis* (Echinodermata: Asteroidea): allozyme differentiation among Japanese, Russian, and recently introduced Tasmanian populations. *Mar Biol* 124:99–109. doi:10.1007/BF00349151
- Weir BS, Cockerham CC (1984) Estimating {F}-statistics for the analysis of population structure. *Evolution* 38:1358–1370

- Westfall KM, Gardner JPA (2010) Genetic diversity of Southern hemisphere blue mussels (Bivalvia: Mytilidae) and the identification of non-indigenous taxa. *Biol J Linn Soc* 101:898–909. doi:[10.1111/j.1095-8312.2010.01549.x](https://doi.org/10.1111/j.1095-8312.2010.01549.x)
- Whitehead A, Crawford DL (2006) Neutral and adaptive variation in gene expression. *Proc Natl Acad Sci USA* 103:5425–5430. doi:[10.1073/pnas.0507648103](https://doi.org/10.1073/pnas.0507648103)
- Whitlock MC, Lotterhos KE (2015) Reliable detection of loci responsible for local adaptation: inference of a null model through trimming the distribution of  $F_{st}$ . *Am Nat* 186:S24–S36. doi:[10.1086/682949](https://doi.org/10.1086/682949)
- Whitlock MC, McCauley DE (1999a) Indirect measures of gene flow and migration:  $F_{ST}$  not equal  $1/(4Nm + 1)$ . *Heredity* 82:117–125. doi:[10.1038/sj.hdy.6884960](https://doi.org/10.1038/sj.hdy.6884960)
- Whitlock MC, McCauley DE (1999b) Indirect measures of gene flow and migration:  $F_{ST}$  not equal  $1/(4Nm + 1)$ . *Heredity* 82:117–125
- Wiedenmann J, Baumstark A, Pillen TL, Meinesz A, Vogel W (2001) DNA fingerprints of *Caulerpa taxifolia* provide evidence for the introduction of an aquarium strain into the Mediterranean Sea and its close relationship to an Australian population. *Mar Biol* 138:229–234. doi:[10.1007/s002270000456](https://doi.org/10.1007/s002270000456)
- Williams GC (1975) Sex and evolution. Princeton University Press, Princeton
- Wray NR, Yang J, Hayes BJ, Price AL, Goddard ME, Visscher PM (2013) Pitfalls of predicting complex traits from SNPs. *Nat Rev Genet* 14: 507–515 doi [10.1038/nrg3457](https://doi.org/10.1038/nrg3457). <http://www.nature.com/nrg/journal/v14/n7/abs/nrg3457.html> - supplementary-information
- Yamashita M, Komatsu M, Kijima A (2005) Genetic variability and geographical population structure in coastal starfish *Asterias amurensis* Lutken around Japan estimated by Isozyme analysis, pp 99–108
- Yoder JB, Stanton-Geddes J, Zhou P, Briskine R, Young ND, Tiffin P (2014) Genomic signature of adaptation to climate in *Medicago truncatula*. *Genetics* 196:1263–1275. doi:[10.1534/genetics.113.159319](https://doi.org/10.1534/genetics.113.159319)
- Zhan A, Briski E, Bock DG, Ghabooli S, MacIsaac HJ (2015) Ascidiaceans as models for studying invasion success. *Mar Biol* 162:2449–2470. doi:[10.1007/s00227-015-2734-5](https://doi.org/10.1007/s00227-015-2734-5)
- Zhang L, Wang X, Liu T, Wang G, Chi S, Liu C, Wang H (2015) Complete plastid genome sequence of the brown alga *Undaria pinnatifida*. *PLoS ONE*. doi:[10.1371/journal.pone.0139366](https://doi.org/10.1371/journal.pone.0139366)
- Zhao X, Yu H, Kong L, Liu S, Li Q (2014) Comparative transcriptome analysis of two oysters, *Crassostrea gigas* and *Crassostrea hongkongensis* provides insights into adaptation to hypo-osmotic conditions. *PLoS ONE* 9:e111915. doi:[10.1371/journal.pone.0111915](https://doi.org/10.1371/journal.pone.0111915)