Review

Conservation genetics in transition to conservation genomics

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Over the past twenty years conservation genetics has progressed from being mainly a theory-based field of population biology to a full-grown empirical discipline. Technological developments in molecular genetics have led to extensive use of neutral molecular markers such as microsatellites in conservation biology. This has allowed assessment of the impact of genetic drift on genetic variation, of the level of inbreeding within populations, and of the amount of gene flow between or within populations. Recent developments in genomic techniques, including next generation sequencing, whole genome scans and gene-expression pattern analysis, have made it possible to step up from a limited number of neutral markers to genome-wide estimates of functional genetic variation. Here, we focus on how the transition of conservation genetics to conservation genomics leads to insights into the dynamics of selectively important variation and its interaction with environmental conditions, and into the mechanisms behind this interaction.

The conservation genetics paradigm

A central idea in conservation genetics is that small, isolated populations can be threatened for the following genetic reasons [1,2]. The dynamics of genetic variation over space and time in a (set of) population(s) containing only a few individuals is expected to be strongly dominated by random genetic drift and inbreeding.

Genetic drift – the random fluctuation of allele frequencies over time – will lead to random loss and fixation of alleles; owing to the random nature of genetic drift adaptive alleles can be lost and deleterious alleles can become fixed in the population. Inbreeding, in the context of conservation genetics, is often invoked as biparental inbreeding, and leads to an increased frequency of homozygotes in the population.

At least three important consequences result from these effects of genetic drift and inbreeding. First, the increased homozygosity and increased frequency of deleterious alleles will often lead to inbreeding depression – an average reduction of individual fitness – and thus might

Glossary

Adaptive potential: the capacity of a population to adapt to changing environmental conditions in an evolutionary manner that involves changes in allele frequencies in the population's genetic pool.
AFLP: amplified fragment length polymorphism. A type of neutral marker, based on a combination of restriction site variation and PCR amplification.
Allee effect: a decrease in fitness due to low density of individuals in a population. Allee effects are often understood in terms of the unavailability of suitable mates in low-density populations.
Biparental inbreeding: inbreeding as a consequence of relatedness. In a population with on average relatively high levels of relatedness even a random cross involves a certain level of inbreeding. Average relatedness is expected to be higher in small populations.
Coalescence theory: a retrospective model of population genetics. It employs a sample of individuals from a population to trace all alleles of a gene shared by all members of the population back to a single ancestral copy known as the most recent common ancestor (MRCA). The statistical properties of this backward procedure allow estimations of, among others, effective population size, mutation rates and gene flow.
Effective population size (N_e): the number of breeding individuals in an idealized population that would show the same amount of dispersion of allele frequencies under random genetic drift or the same amount of inbreeding as the population under consideration. It is a basic parameter in many models in population genetics. The effective population size is usually smaller than the absolute population size (N).
Fitness: absolute fitness for a genotype can be calculated as the product of the relative survival time and average fecundity. Relative fitness is quantified as the average number of surviving progeny of a particular genotype compared with average number of surviving progeny of the fittest genotype.
Gene flow: also known as gene migration, gene flow is the transfer of alleles of genes from one population to another. Migration into or out of a population can be responsible for a marked change in allele frequencies.
Genetic drift: in the evolutionary process of random change in the allele frequencies (or gene frequencies) of a population from one generation to the next due to sampling effects. The extent of genetic drift is inversely related to effective population size.
Inbreeding depression: the reduction in fitness of offspring of an inbred cross, often sib-mating (in animals) or selfing (in plants), compared to the fitness of offspring of an outcross within the same population.
Markov Chain Monte Carlo: a statistical simulation technique where outputs of generation n serve as input for generation $n+1$ in a randomly drawn dataset. Often used to analyze parameter spaces of complex statistical models.
Metapopulation: a population of populations; a series of populations dynamically connected via dispersal.
Methylation-sensitive AFLP: MS-AFLP is a regular AFLP approach using two isoschizomers (*Hpa*II and *Msp*I) that recognize the same restriction site (CCGG) but differ in their sensitivity to methylation at the restriction site. The method is used to screen for genome-wide methylation variation.
Microarray: a DNA microarray is a high-throughput technology used in molecular biology and in medicine. It consists of an arrayed series of thousands of microscopic spots of DNA oligonucleotides, called features, each containing picomoles of a specific DNA sequence.
Microsatellite: a PCR-based neutral marker that exploits variation in the number of repetitive elements at a particular locus. Also known as SSR (simple sequence repeats).

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Outbreeding depression: the reduction in fitness of offspring of an outcross between two distinct populations compared to the fitness of offspring of an outcross within a single population.
Phylogeography: an approach to analysis of marker patterns where phylogenies are mapped onto geographical position of the populations/species, allowing colonization histories to be inferred.
Phenotypic plasticity: the ability of a genotype to change in phenotype in response to environmental differences.
RNA-seq: an approach where mRNA is collected, reverse-transcribed into cDNA, and directly sequenced (sometimes after normalizing the sample to reduce the level of highly expressed RNAs such as ribosomal RNA) using 454 or, more often, Solexa sequencing procedures.

decrease the short-term viability of a population. Second, the loss of genetic variants will compromise the evolutionary adaptive potential of a population, and can thereby reduce the long-term viability of a population, especially in changing environments. Third, small, isolated populations will become increasingly genetically divergent as a consequence of the independent action of genetic drift in each population. Crossing individuals between these populations, for instance in restoration programs that aim to enhance gene flow between previously isolated populations (e.g. by making corridors in the landscape so that populations can mix more easily), might then lead to outbreeding depression (Glossary).

Although these processes are predicted to impact upon small populations on the basis of theoretical considerations derived from population genetic theory [1], the development of PCR technology has stimulated conservation genetics to become a well-established empirical discipline. The availability of easy to apply and highly variable neutral markers including microsatellites and amplified fragment length polymorphisms (AFLPs; Glossary) (Figure 1) has allowed the relationship between population size and the level of genetic variation and inbreeding to be tested. Many studies have addressed whether population size is positively correlated with the level of genetic variation within populations, and is negatively correlated with the level of inbreeding within populations, as predicted by the conservation genetics paradigm. However, several questions remained unresolved because of the limitations of the use of a small number of neutral genetic markers. The use of neutral markers precluded assessment of selectively important genetic variation, and made it difficult to investigate the interaction between genetic and environmental impacts on fitness. Moreover, the markers were used in a correlative approach, trying to find associations between marker diversity and population size on the one hand, and fitness on the other, but this provided no means to study the mechanisms behind important processes such as inbreeding depression and local adaptation.

The current rise of genomic approaches and the availability of genomic resources, even for ecological model species, now offer new opportunities for tackling these unresolved issues (Figure 2). Therefore, after first outlining the achievements of conservation genetics over the past decades, we identify four main unresolved questions. (i) To what extent is selectively important genetic variation affected in small, isolated populations? (ii) What are the mechanisms that connect (the dynamics of) genetic variation with fitness and adaptation? (iii) How, and to what extent, do genetic and environmental effects interact when influencing genetic variation, fitness and adaptation in small, isolated populations? Finally, (iv) how do inbreeding and genetic drift affect the activity of genes (as opposed to their effects on sequence variation)? We describe how genomic approaches might be able to help solve these questions and can be instrumental in obtaining important new insights in the role of genetics in conservation.

Developments in conservation genetics

Over the years conservation genetics has tremendously improved our insight into processes that are associated with habitat fragmentation and small-population size.

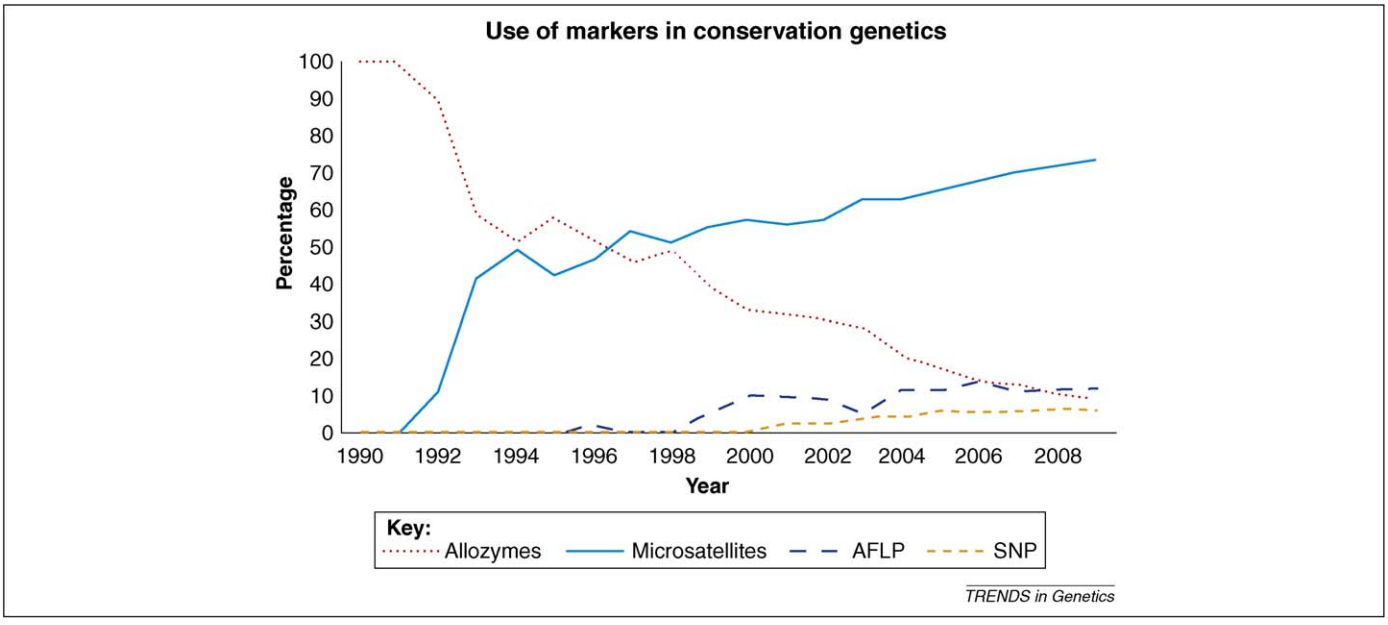

Figure 1. The growth of conservation genetics. Conservation genetics is a growing field as revealed by the increasing numbers of papers published on this topic over the past ten years. The graph shows the frequency of papers containing the term 'conservation genetics' and the respective name of the marker in a Web of Science search (http://thomsonreuters.com/products_services/science/science_products/a-z/web_of_science) plotted against time. The total number of papers has increased from four in 1990 to 682 in 2009.

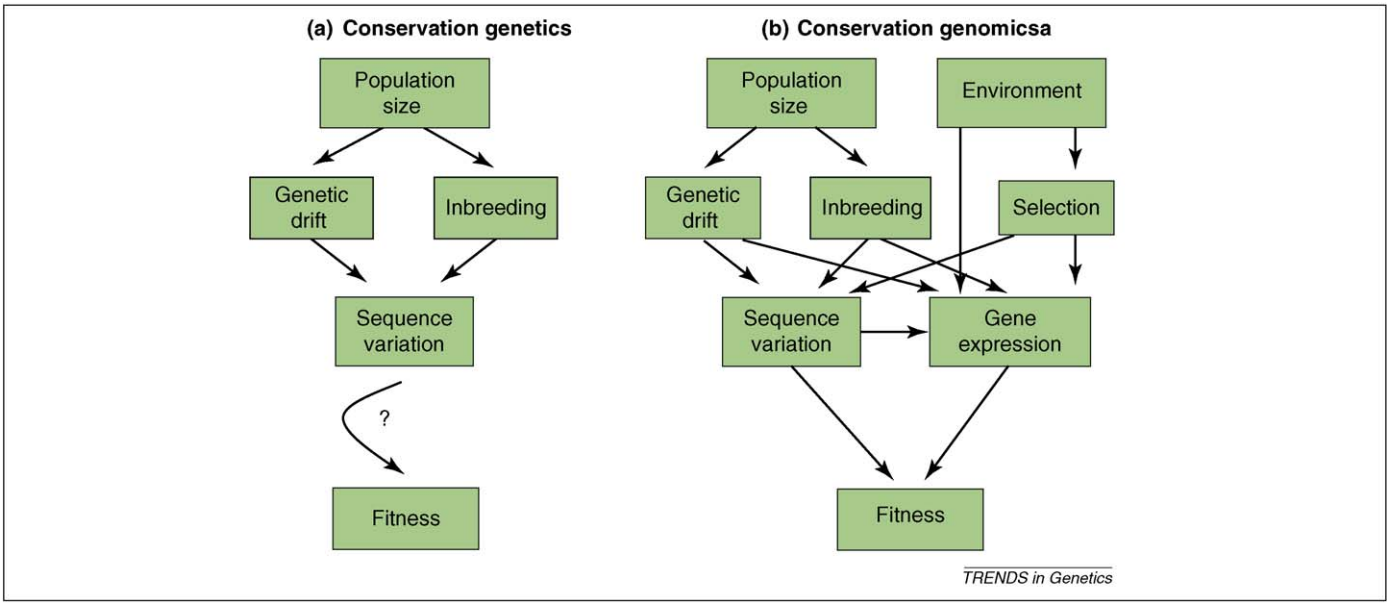

Figure 2. Schematic representation of **(a)** the conservation genetics approach and **(b)** the conservation genomics approach. Conservation genetics is characterized by assessing relationships between population size and neutral sequence variation; the relationship with fitness is based on the assumption that neutral marker variation is indicative of selectively important variation. By contrast, conservation genomics assesses relationships between population size and both neutral and selectively important variation, both in terms of sequence variation and gene expression variation, and thereby incorporates potential effects of selection. Conservation genomics also incorporates the influence of environmental conditions on sequence variation (via selection) and on gene expression variation. The relationship with fitness is potentially based on mechanistic inferences.

These achievements can be summarized into three distinct categories.

First, the massive application of markers such as microsatellites has generated a huge and ever-increasing database on the effects of small population size on the extent and distribution of neutral genetic variation. We know that, even though exceptions can be found, small population size is generally a good predictor for low levels of allelic variation and increased levels of homozygosity [3]. The analysis of the distribution of this variation over space is now a routine part of defining evolutionarily significant units (ESU) and of estimating the effects of landscape changes on the dispersal and gene flow in all kinds of species [4].

Second, in addition to the major advances made in empirical approaches, conservation genetics has also made significant progress in designing efficient methods to translate neutral marker patterns in space into inferences about demography, gene flow, effective population size (N_e), metapopulation structure, and phylogeography [5]. The information contained in marker patterns is in many conservation genetic studies one of the best ways, if not the only way, of obtaining information about the current (and past) status of threatened populations and possible management strategies to alleviate these threats. For example, comparing observed heterozygosities between large and small populations of a species can give insights into the impact of inbreeding on small populations of these species, whereas analyzing genetic differentiation between populations in a landscape leads to inferences about the level of isolation between them. Conservation genetics has stimulated the application of coalescence theory, of maximum likelihood approaches, of Markov Chain Monte Carlo techniques and of the use of Bayesian statistics [6]. Clear and strong examples are the spatial analysis of allelic patterns using Bayesian clustering techniques such as STRUCTURE, BAPS and GENELAND [7] and the elucidation of paternity and kinship relations [8,9].

Third, the conservation genetics approach has provided very important insights into processes that are associated with the conservation genetics paradigm, but are equally important for other areas of evolutionary biology. The most prominent example is the study of inbreeding depression, that is considered to be an important phenomenon in conservation genetics, and is thought to be the mediator between loss of genetic variants through drift and the effects of this loss on the probability of extinction. At the same time inbreeding depression plays an important role in models of evolution in mating systems in plants and animals [10,11]. We know that inbreeding depression is environment-dependent, is genotype- and population-specific, and can affect different life-history traits in different and non-correlated ways [12]. However, to what extent inbreeding depression affects the persistence of populations and species is still an open question, despite a few isolated well-studied examples. In field populations of the butterfly *Melitaea cinxia* a correlation between the level of inbreeding in the population and the probability of extinction has been demonstrated [13]. In laboratory populations of *Drosophila melanogaster*, inbred lines had higher rates of extinction than non-inbred lines; this effect was most obvious under stressful environmental conditions [14].

What are the major unresolved questions in conservation genetics?

Despite considerable progress, several questions in conservation genetics are still unresolved. Here we discuss those that we consider to be the main four.

First, it is at present unclear how fitness-related functional genetic variation is affected by the same processes that have been demonstrated to affect neutral marker variation. That is: is there a correlation between population size and level of non-neutral genetic variation? The conservation genetics paradigm inherently predicts a positive correlation across species between population size and the level of genetic variation. This correlation has been demonstrated in meta-analyses of animal and plant studies [3,15]. In addition, the predicted correlation between population size and average individual fitness has been demonstrated [3]. This correlation is likely to be caused by genetic processes, but is additionally affected by other, non-genetic, processes that are associated with population size, such as Allee effects, disruption of species interactions, or the deterioration of local habitat quality [16,17].

The effect of genetic drift is proportional to the effective population size N_e. If N_e is small it is expected that even strongly selected markers will behave essentially as neutral variants (provided that the product of N_e and s, the selection coefficient, is $<<1$) and that the genetic variance in neutral markers will coincide with the genetic variance in non-neutral markers. By contrast, if N_e is large then the correlation between neutral marker diversity and strongly selected markers is weak and becomes even weaker in expanding or declining populations [18]. Thus the correlation between N_e and genetic variation will be strongest for neutral markers, and much weaker for strongly selected markers [19].

Unfortunately, most of the conservation genetics studies conducted so far have used near-neutral markers, and thus empirical tests of the effect of small effective population size on functionally important genetic variation are virtually lacking. It is also unclear how representative the estimates of genetic variation are for genome-wide genetic variation, when estimated based on a small number of microsatellites. Few studies have compared microsatellite and single-nucleotide polymorphism (SNP) diversity within the same samples, with equivocal results. A correlation was found in Atlantic salmon [20], but no correlation was found at the individual level in wolves and North-American coyotes [21]. At the same time in this last study a high correlation was found at the population level, implying that the representativeness of neutral marker patterns for overall genetic variation is different at different organizational levels. Clearly, we are in need of methods that assess genome-wide genetic variation, and specifically target selectively important variation in a conservation genetics context, to test whether small, isolated populations might have lowered adaptive potential.

Second, although conservation genetics has made great progress in predicting and explaining the dynamics of neutral genetic variation, the mechanisms that connect this dynamics with adaptation and fitness are unknown. Inbreeding depression, one of the central phenomena in conservation genetics, has unknown genomic causes, even if we have insight into the types of mutations involved [22]. However, to make predictions of the consequences of small population size for fitness we need a much better understanding of these genomic causes. It is clear that it is not adequate to incorporate inbreeding depression into demographic analyses as a single value for each particular population, because inbreeding depression is a complex and highly dynamic phenomenon that varies between populations, genotypes, life-history stages and environments [2,12]. We therefore need to investigate how many genes and/or genomic pathways are involved in inbreeding depression, the contribution of each to the inbreeding depression phenotypes, and how the activities of these genes respond to ontogenetic and environmental variation. Is inbreeding depression a consequence of the increase in the frequency of deleterious alleles at a random set of genes, or are the same genes or gene pathways involved across populations [12,23]? Are there similarities at the gene expression level with processes such as ageing that also reflect a decrease in fitness? Does the rate of inbreeding matter, and is more variation maintained with slow compared with fast inbreeding [24]? Answering these and similar questions is vital for a full understanding of the role of inbreeding depression in conservation genetics.

Third, conservation genetics has focused almost exclusively on the effects of drift and lack of gene flow associated with habitat fragmentation. However, the extinction of populations will be influenced by more – or even more important – processes such as environmental degradation, climate change and disruption of species interactions [25,26]. The extent of the interaction between these non-genetic effects and genetic processes, summarized by genotype-by-environment (G x E) interactions, is as yet largely unknown. How populations adapt to a changing environment, and what effects drift and inbreeding have on this process, are to a great extent unknown [27]. Detailed insight into the role of local adaptation in a network of populations is of the utmost importance in a conservation genetics context; strong local adaptation would call for management scenarios directed towards individual populations rather than scenarios that promote the dispersal and coherence of the metapopulation [28,29]. A further question is to what extent phenotypic plasticity interacts with the effects of drift and inbreeding [30]. On the one hand, in populations depleted of genetic variation, phenotypic plasticity can allow short-term adaptation to changing environments, but on the other hand it might increase phenotypic variance and thereby reduce the evolutionary response to selective pressure [31,32]. In the hermaphroditic snail *Physa acuta* inbreeding strongly reduced the expression of predator-induced adaptive plasticity [33]. In general, however, we need to know much more about how drift and inbreeding might affect the potential for phenotypic plasticity.

Finally, although conservation genetics has focused on the dynamics of sequence variation, much less, if any, attention has been given to variation in gene expression. The way in which gene expression is altered by changes in cis- or trans-regulatory mechanisms as a function of drift and inbreeding is largely unknown. However, it has been argued that gene expression might be closer to phenotypic trait variation than gene sequences [34,35]. Environmentally induced stress, such as that imposed by drought, increased temperature or lack of food or nutrients, can result in changes in genomic pathways, both in plants [36] and in animals [37]. Kristensen *et al.* [38] demonstrated

major changes in gene expression profiles in inbred lines of *D. melanogaster* compared with those in outbred lines, but we are only starting to understand how gene expression responds to drift and inbreeding, both factors that can be considered to constitute genetic stress. The regulation of gene expression often has an epigenetic component, where DNA methylation and histone modification can result in phenotypic variation, even in the absence of sequence variation. However, we do not know how epigenetic mechanisms, that are frequently induced by environmental factors, respond to genetic drift and, especially, to inbreeding.

How can genomics advance conservation genetics?

In the past few years there has been a growing tendency to adopt the techniques, approaches and data of functional genomics and apply them to natural populations in natural environments [39–41]. This has been stimulated by the insight that the gene function can only be defined in close relation to the environment in which it is measured. Thus in most cases it is an open question whether the achievements of functional genomics, obtained in model organisms (*Arabidopsis thaliana*, *Caenorhabditis elegans*, *Mus musculus* and *D. melanogaster*), in a limited set of well-defined genotypes and under strictly controlled environmental conditions, apply to natural situations. For instance, artificial selection for heat resistance of *D. melanogaster* lines led to unpredicted fitness responses of these lines in field trials [42]. The need to expand genomic research out of the lab to natural populations and from model to non-model organisms has been stressed previously [39,40].

There are several challenges to developing an ecological and evolutionary approach to genomics. These involve changing from genomic model species, that have ample genomic resources and tools available, to ecological model species, for which these resources and tools are generally absent. They also involve moving from a lab or greenhouse environment, where environmental variation is kept at a minimum, to natural environments, where genetic and environmental variation are a fact of life and can make it more difficult to discern genetic effects from environmentally induced effects [43,44]. Finally, adopting an ecological and evolutionary functional genomic approach [39], for sake of brevity often referred to as ecogenomics, will force techniques and approaches originally designed for detailed studies at the individual (and even cell) level to be used in population-wide studies [45].

Conservation genetics is clearly ready to take up the possibilities and challenges offered by incorporating genomics into its research. Attempts to start ecogenomic research programs in a conservation context are rapidly increasing in number [46–49]. Instrumental in this development is the availability of next-generation sequencing technologies such as 454 pyrosequencing (Roche), Solexa sequencing-by-synthesis (Illumina), and SOLiD (sequencing by oligo ligation and detection; Applied Biosystems) (technical details can be found in Refs [50,51]).

In a conservation genetic context, most species of interest do not have sequence resources available. Currently the most feasible way to obtain these sequences is to sequence of a pool of mRNA samples, preferably from several tissues and several individuals, usually with 454 technology. *De novo* assembly of the resulting 500000+ sequence reads will typically result in 30000 to 40000 contigs. These contigs can be blasted against the phylogenetically closest genomic model organism, or at least a species with sufficient annotated sequences available, to annotate the contigs; a nice example can be found in Ref. [48]. Because the pool consisted of several individuals, the overlapping reads in a scaffold will provide information about the presence of SNPs. A typical 454 run will result in thousands of SNPs [50]. The run typically will also yield hundreds of expressed sequence tag (EST)-related microsatellites (e.g. Ref. [51]).

Within a relatively short time span, and depending on facilities and finances, an ecological model species can thus be 'lifted up' to an information level that is sufficient to start a conservation genomic research program. Examples include the metapopulation-model species *M. cinxia* [48], the lake sturgeon *Acipenser fulvescens* [52], the coral *Acropora millepora* [53], and the tree *Eucalyptus grandis* [54], and the number of examples is increasing rapidly.

Population genomics

Sequence information obtained in this way can be used in a conservation context in two separate ways. First, the SNP and EST-SSR (simple sequence repeat) markers can be used in studies of selection. SNPs in particular will cover a large part of the genome, with much larger coverage than the traditional microsatellites and AFLPs. These markers are first used to obtain a much more representative view on the genetic variation within individuals, populations and metapopulations. This allows higher resolution inferences about demography, gene flow, inbreeding and population history. Second, these markers can be instrumental in a study of 'footprints of selection'. They can be applied in a population genomic approach (Box 1) where the deviating distribution pattern for individual markers can be indicative of past selection at loci linked to the marker [55,56]. Although identifying selective effects in natural populations is a challenge [27,57], performing genome scans and association studies in natural populations is a real and valid option [35,58], also because new statistical methods provide increased power [59]. Even so, identifying the actual genes under selection is still a major challenge. However, the first priority of conservation genomics is finding areas, and markers, in the genome that are under selection. Comparison of spatial and temporal patterns of neutral markers, and markers that were identified in a genome scan to have selective value, would allow the disentanglement of genetic drift and selection effects. This opens the way to the study of the effects of habitat quality, as affected by local deteriorating factors and by global factors such as climate change, in close conjunction with genetic effects imposed by habitat fragmentation; such studies could reveal the net effects of changing environments on the different regions of the genomes.

Population transcriptomics

The second application of transcriptomic sequences derived from high-throughput sequencing is to use these sequences as a reference for mapping transcripts. Until

Box 1. Population genomic approaches available for conservation.

Population genomics, when narrowly defined, aims at using genome-wide sampling to identify and to separate locus-specific effects (selection, mutation and recombination) from genome-wide effects (genetic drift, inbreeding and gene flow) [55]. Conservation genetics has until now focused on genome-wide information to estimate parameters of population and metapopulation demography and of phylogenetic and phylogeographic history. Population genomics emphasizes the importance of a transition to a conservation genomics perspective in three ways: (i) population genomics approaches rely on the simultaneous use of many markers covering the entire genome (rather than using 10–20 markers, as is routine in conservation genetics), (ii) population genomic approaches allow neutral markers to be distinguished from non-neutral markers, and (iii) based on the assessment of neutrality and non-neutrality more reliable estimates of demography and history can be made by excluding the non-neutral markers from the analysis.

Population genomics comes in various forms but they all share the same basic steps [55]: (i) sampling of many individuals, (ii) genotyping of many markers, (iii) performing statistical tests to search for outlier loci that might indicate non-neutrality. Several tests are available that compare genetic variance between loci within or between populations with model predictions [59,73,74]. Population genomic approaches available for conservation have been discussed by Kohn *et al.* [71] and include candidate-gene approaches, genome scanning, and association mapping. The potential of next-generation sequencing now brings a new approach within close reach of conservation genomics.

Population transcriptomics

In this approach differences in gene expression, rather than in gene sequence, are exploited to identify evolutionarily important genetic variation. The method has typically involved the use of microarrays [75] containing thousands of genes from the focal species, allowing simultaneous assessment of their expression in response to environmental and genetic factors [23,68]. The downside of microarrays is however that they are relatively species-specific tools and their use can only be extended to phylogenetically close species ('genomic enabled species', *sensu* Kohn *et al.*, Ref. [71]). Modern techniques, such as RNA-seq [60], can be applied *de novo* to any species and thus are flexible tools in a conservation context. The population transcriptomic approach might be able to: (i) identify genes responsive to both environmental and genetic stresses [23,36–38,68], and (ii) identify genes and gene pathways as candidates for (disruption of) local adaptation, by distinguishing between the effects of drift and selection [76]. This field is still in its infancy, but is rapidly developing in ecogenomics research. Conservation genomics could both profit from this development and make a significant contribution to it.

now conservation genetics has focused on sequence variation. A population transcriptomic approach would enable the study of variation in gene activity as a function of habitat fragmentation and environmental change. In as far as evolutionary changes are actually based on changes of gene expression regulation, rather than on changes in the actual genes themselves [34], population transcriptomics might provide a completely new view on the effects associated with small, isolated populations. Methods for population transcriptomics in conservation genomics have relied on the use of heterologous microarrays [75] but now increasingly rely on high-throughput RNA sequencing (RNA-seq; [50,60]) where the output of an RNA-seq run is mapped on the reference EST data to estimate transcript levels for each EST. At the same time, each RNA-seq run will provide sequences that can be assembled with the reference ESTs, thereby constantly expanding the reference genome.

Conceptually the results differ from the sequence variation approach that characterizes conservation genetics in that the results are by necessity a function of both genetic and environmental factors. Thus, whereas the use of SNPs and EST-SSRs and their application in genome scans and association studies is an expansion of the conservation genetic concept, population transcriptomics is a conceptual innovation towards conservation genomics. The transcriptomic approach will allow the study of the mechanisms of maladaptation due to habitat fragmentation effects, and will allow deeper understanding of complex phenomena including inbreeding depression [12], for instance by identifying causal genes for inbreeding depression in various life-history stages, metapopulation dynamics [48], for instance by finding genes associated with dispersal or dispersal behavior and monitoring their activity as function of local and regional environments, and also phenotypic plasticity [61] and interactions between genetics and environment (G x E) [62,63] by monitoring gene expression as function of (epi)genetic and environmental factors.

Population transcriptomics is only the beginning of this conservation genomics approach. Finding effects of habitat fragmentation and small population size can be expected also at the proteome and metabolome level, and population proteomics [64] and even population metabolomics [12,65] approaches are currently starting to be part of the 'omics' family. In addition, fields that aim to understand the mechanisms of transcription regulation are progressing rapidly. The most prominent of these fields is epigenomics (Box 2) that studies how environmental cues can lead to changes in the regulation of gene expression via specific epigenetic processes including DNA methylation and histone modifications. As such the field could provide a framework for a conservation genomics research program that tries to reach a deeper understanding of how environmental change and genetic erosion might interact.

Challenges and limitations

As in any new emerging field, conservation genomics will have to face several challenges and limitations. Most importantly, trying to understand the relationship between genes and adaptation, a long-standing goal of population genetics, evolutionary biology and functional genomics alike, is a notoriously difficult enterprise. A first requirement is that studies will have to be conducted from many angles including population genetics, ecology, demography, environmental biology and bioinformatics. Conservation genomics will have greatest chances of success only in those cases where large multidisciplinary research programs are backed up by genomic research [66]. Therefore, conservation genomics should aim for a limited number of conservation model species instead of performing population genomics and population transcriptomics in any threatened species. In that sense conservation genomics focuses primarily on the general principles that relate small population size and population isolation to the way genetic and genomic processes affect extinction, rather than trying to assess the conservation status of each single species or population.

Box 2. Epigenetics and conservation

Until recently a key assumption in biology has been that DNA polymorphisms are the only source of heritable variation in natural populations. It was assumed that the ability of species to adapt to environmental change depends on the presence of DNA sequence variation, and that evolutionary change and adaptation were impossible without it [77].

However, there is growing evidence that heritable variation in ecologically important phenotypic traits can also be caused by variation in epigenetic modifications of the genome, even in the complete absence of DNA sequence variation [78]. The heritable nature of such epialleles [79] is based on the maintenance of chromatin features such as DNA methylation and the modification of DNA-associated proteins such as histones that regulate the activity of genes and thus ultimately determine gene expression and phenotype. Although these modifications are determined by antagonistic enzymatic activities, and thereby are in principle reversible [80], they can achieve surprising stability upon somatic and sexual propagation and change phenotypic variation of subsequent generations.

Recent studies indicate that patterns of chromatin modifications can vary between individuals and populations of the same species [81], and that at least part of this variation is heritable and independent of DNA sequence variation [82,83]. Moreover, as with DNA sequence variants, induced chromatin changes can in some cases become inherited by future generations [84,85]. Therefore, these differences and any additional induced epigenetic and inherited change – both stable or reversible – might provide an important and versatile mechanism for plants and animals to rapidly adapt to environmental change [85–87].

Epigenetics is likely to be an important field in conservation genomics. In populations that have been depleted of genetic sequence variation, environmentally induced epigenetic marks that are stably inherited by subsequent generations could potentially allow organisms to adapt to changing environments. It is at present unclear in how far this mechanism could provide rescue for genetically depauperated populations, but the phenomenon deserves attention. Which environmental cues result in which epigenetic marks? Which of these marks are inherited by subsequent generations? And, if inherited, for how many generations can environmentally induced marks be traced after the environmental cue has been removed? How do genetic mechanisms such as genetic drift and inbreeding affect the responsiveness of DNA methylation mechanisms? What is the relative contribution of epigenetic mechanisms compared to the evolution of gene sequences? The tools to study these questions at the population level are increasingly becoming available, and also for non-model species [88]. Thus incorporating an epigenetic approach in conservation genomics (and in other fields of ecology and evolutionary biology) is likely to result in exciting new insights in the way evolution might lead to adaptation.

Although genome scans and association studies can be performed in ecological model species, the reliability of these approaches increases with the number of samples analyzed. Environmental variation in natural populations is larger than in laboratory or greenhouse conditions, and consequently more samples are needed to find significant associations between markers and phenotypic traits [67]. In addition, it is expected that in small populations the effects of genetic drift can be very prominent, thereby complicating discovery of 'footprints of selection' in these small populations. A more viable strategy would probably be to undertake genome scans in larger populations of the focal species (if available), and then try to use markers with a selective signal as 'candidates' in the smaller populations.

Even if significant associations are found between a marker and a trait, pinpointing the responsible gene is not trivial. There are relatively few examples of studies that have been able to discover the actual gene behind an observed quantitative trait locus (QTL). Slate [67] stated that finding the gene behind the QTL in natural populations is difficult unless very convincing candidate genes are available. However, in conservation genomics the first objective is not necessarily to find the gene. Instead, the main goal is typically to find markers associated with certain phenotypic traits and to compare their distribution patterns within and between populations against the patterns of supposedly neutral markers.

Population transcriptomics also presents several challenges. For instance, it is not trivial to distinguish between cause and consequence genes in the transcriptional profiles of inbred versus outbred individuals [12]. Although many genes change their expression as a consequence of inbreeding [38,68], a search for QTLs for specific traits showing inbreeding depression does not reveal many of the loci involved [69]. This exemplifies the problem of separating primary from secondary effects in that expression changes in some genes can automatically alter the relative expression levels in many other genes even if these genes are not direct targets for selection. Perhaps the most important challenge when trying to make inferences about population processes is how to deal with transcript variation that is highly tissue-specific and in many cases highly dynamic on a temporal scale [45]. Part of the solution is familiar to any population biologist: biological replication. The practice in molecular biology – to use only a few measurements of gene expression, and to concentrate on technical rather than biological replication – will not be viable in conservation genomics. But whereas large designs with the level of replication that ecologists are used to are currently not feasible owing to the high costs of performing a transcriptomic analysis, this limitation will most likely be overcome with technical advances and the anticipated reduction in cost in the near future. However, the highly dynamic and tissue-specific nature of transcriptomic data at the population level is a serious challenge, and demands careful design and standardization.

Concluding remarks and future prospects

We have discussed the potential of incorporating a genomic approach into conservation genetics. The need to adopt a conservation genomic approach is evident from a long-term dilemma in conservation genetics: do the patterns observed with neutral markers indicate patterns of adaptive or detrimental variation? Or, to put it in other words, are neutral marker studies, that deliver much information about genetic drift, inbreeding, gene flow, and demography, relevant for assessing the fitness and adaptive potential of populations? In 2001, Hedrick [70] argued that this might not be generally the case. In 2006, Kohn *et al.* [71] were the first to argue that the use of extensive genome sequence information would provide valuable insights concerning this dilemma. Here we have expanded on their arguments, based on the ever-growing possibilities of genomic techniques and approaches. Not only will genomic techniques allow a genome-wide estimation of both selec-

Box 3. Conservation genomics in wolf populations

Populations of grey (*Canis lupus*) and red (*Canis rufus*) wolves in North America and Europe have been the subject of conservation genetic studies for more than a decade. Because wolves are phylogenetically close to domestic dogs they can be considered to be 'genomic enabled species' [89]. Research in wolves provides a good case for demonstrating the transition from conservation genetics to conservation genomics.

Many studies using microsatellites have been conducted in the conservation genetics context to estimate genetic variation, population structure, gene flow, demographic history, and hybridization events [90–92]. Population genomic approaches are increasingly being applied in wolf research. Candidate gene approaches have been performed using MHC genes [93] or the K-locus that is involved in color polymorphism [94]. For instance, it was found that heterozygosity at the *DRB1* gene of the MHC complex in red wolves was eight times higher in a gene region containing important binding sites than in sites outside this area, suggesting balancing selection [93]. The frequency of the dark color-morph associated with an allelic variant of the K-locus was much higher in forest populations, suggesting adaptive significance of the allele (however, see Ref. [95]).

Increasingly, genome-wide markers, including indel-polymorphisms [96] and SNPs [97], are being developed and used for studies of demographic history [98], and hybridization and introgression events [99]. Genome scan studies identified 21 loci potentially affected by directional selection in a population of Scandinavian wolves [100]. A population transcriptomic study identified 482 ESTs (out of 2980 tested) that were differentially expressed between free-ranging and confined populations of red wolves in North America; an over-representation of genes involved in stress responses, including insulin signaling and tryptophan metabolism pathways was discovered among these 482 ESTs [101]. The expression data were analyzed by principal component analysis and the contribution of genetic and environmental factors was estimated. Of the first axis, 34.4% was explained by genetics and 41.6% by habitat, whereas at the second axis 70.4% was explained by genetics and only 2.9% by habitat. This demonstrates that population transcriptomics potentially is an approach to treat genetic and environmental effects on genetic variation and gene expression in a truly integrated way.

tively important and neutral variation, but they will also allow the detailed study of the genomic mechanisms that translate the effects of small population size into effects on adaptation. Conservation genomics will expand our perspective from effects on sequence variation to effects on gene expression, and thereby opens the door widely for an integrated approach towards assessing the relative impact of genetic and environmental threats. Although conservation genomics is still in its infancy, a considerable number of examples can already be found (Box 3). These examples come from 'genomic enabled species' [71], where the threatened species is phylogenetically close to a genomic model species, but increasingly also from non-enabled species as well [48,52–54], where the power of next-generation sequencing leads to the availability of genomic resources even for non-model species.

It is only recently that the immense potential of high-throughput next-generation sequencing, with its promise of rapid development of genomic resources for any threatened species, has attracted attention in a conservation genetics context [66,72]. In the near future it is expected that this potential will lead conservation genomics to major advances in the following areas (Box 4).

First, the use of genome-wide markers will improve estimates of genetic and demographic parameters including individual heterozygosities, genetic distances, past population growth rates, levels of gene flow, and (meta) population structure. Comparison of results obtained with

Box 4. Unresolved questions and possible conservation genomic approaches for tackling them

To assess the impact of habitat fragmentation on selectively important variation

Possible conservation genomic approaches:

1. Use of genome-wide SNPs to obtain a representative estimate of genetic variation
2. Perform a genome scan to distinguish neutral from non-neutral markers
3. Comparison of patterns of neutral (microsatellite, AFLP) and non-neutral (as identified above) variation
4. Undertake an association-mapping approach to find correlations between markers and phenotypic traits important for adaptation
5. Candidate-gene studies can be used to search for frequency changes of alleles in relation to environmental change

To identify genetic mechanisms underlying inbreeding depression

Possible conservation genomic approaches

1. Population transcriptomics can help to identify genes associated with inbreeding depression, in different life-history stages and many genotypes
2. QTL mapping will help to identify genomic regions associated with inbreeding-depression phenotypes
3. Selection experiments on gene-expression phenotypes

To characterize the role of gene–environment (G x E) interactions

Possible conservation genomic approaches:

1. Population transcriptomics can be performed in combination with full factorial experiments to identify genetic, environmental and G x E effects in transcript profiles
2. Perform epigenetic screening, using methylation-sensitive AFLP or high-throughput bisulfite sequencing, of small and large populations in high and low quality habitats.

To identify the role of phenotypic plasticity in the response to environmental challenges

Possible conservation genomic approaches:

1. Epigenetic manipulation experiments (5-azacytidine) to manipulate methylation levels, and study phenotypic effects in relation to population size, inbreeding level and environmental variation
2. Screening of methylation levels as a function of the level of phenotypic plasticity in relation to level of inbreeding

To characterize the effects of habitat fragmentation on gene expression and genomic pathways

Possible conservation genomic approaches:

1. Use microarrays or RNA-seq to screen for changes in genome-wide gene expression profiles in response to inbreeding and population size
2. Screen gene-expression variation in high- and low-diversity populations and genotypes to disentangle direct gene effects from regulatory changes

tens of microsatellites, with results obtained with thousands of genome-wide SNPs in the same set of samples, will tell us how biased the view has been on genetic variation in general, and on the relationship with population size in particular.

Second, the application of population genomics, using thousands of SNPs, will open the way to elucidate the effects of population size and metapopulation dynamics on neutral, detrimental and adaptive genetic variation. It will provide insight into the hypothesized, but until now empirically neglected, effects of small population size on evolutionarily adaptive potential. Population genomics is also the first step towards the incorporation of the important phenomenon of local adaptation into the conservation genetics paradigm, and could lead to an empirically based reappraisal of the role of selection in small populations and metapopulations.

Third, the uptake of transcriptomic, proteomic and metabolomic approaches in a conservation context is a promising step towards understanding the mechanisms underlying adaptation and maladaptation. These approaches will help to provide answers to many questions, such as how many genes, and of what function, are differentially expressed under which conditions in response to inbreeding versus outbreeding? Are these always the same genes across populations? What genes are involved in changes in dispersal behavior? What is the adaptive value of these genes in low as opposed to high dispersal-landscapes? How does environmental degradation and/or climate change interact with genetic effects of habitat fragmentation? By answering these questions conservation genetics will truly transform into conservation genomics, and will have evolved from a correlative discipline that is looking back in time to a causal, mechanistic discipline trying to predict the future dynamics of selectively important variation and potential for adaptation.

These and similar questions are at the core of understanding and managing the dynamics of biodiversity, and therefore conservation genomics presents a challenging but bright future for conservation genetics.

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