Chapter 9
Landscape Genomics: A Brief Perspective

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9.1 Introduction

Landscape genetics is the amalgamation of population genetics and landscape ecology (see Manel et al. 2003; Storfer et al. 2007). In Chapter 17, we discuss landscape genetics and provide two examples of applications in the area of modeling population connectivity and inferring fragmentation. These examples, like virtually all extant landscape genetic analyses, were based on evaluating spatial genetic patterns using a relatively small number of selectively neutral (or nearly neutral) markers. Landscape genomics, on the other hand, is the simultaneous study of tens-to-hundreds of markers, ideally including markers in candidate adaptive genes (genes under selection), with georeferenced samples collected across a landscape. While landscape genomics is, in one sense, simply landscape genetics with lots of data (thus reduced variance and increased precision), the qualitatively different (adaptive, potentially non-independent) nature and analytical approaches associated with these data are different enough to produce a profoundly different field.

In the past year there has been a boom in molecular genetics technology and this has lead to an unprecedented amount of genomics data (Hauser and Seeb 2008;, Mardis 2008; Shendure and Ji 2008; Eid et al. 2009). Consider this: the Human Genome Project, whose goal it was to sequence one human genome cost US$3 billion and took 15 years (Collins et al. 2003), yet today a private company is offering to sequence a whole human genome for $350,000 in 2–3 months

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(http://www.knome.com/home/). Other companies are promising a $100 human genome, produced within one hour, by the year 2012 (http://www.pacificbiosciences.com/index.php). This wealth of genetic information will lead to changes in the way we study animal populations across the landscape.

9.1.1 Population Genomics

To understand what genomics will do for the field of landscape genetics we first need to understand what genomics is, and the difference between selectively neutral markers that are currently being used in landscape genetics, versus adaptive makers that are under selection that will strongly impact the field of landscape genomics. Neutral markers, as we currently understand them, have no direct biological meaning (e.g., they do not influence survival or fecundity, the main components of individual fitness). However, we infer biological activity, such as animal movements, from characteristic genetic patterns derived from neutral markers (Manel et al. 2003).

Population genomics, in contrast, has been defined as the simultaneous study of numerous loci (markers on chromosomes), genes (coding for functional products) or genome regions to better understand the role of evolutionary processes such as genetic drift, selection and migration, that influence variation across genomes and populations (Luikart et al. 2003; Kohn et al. 2006). So, while to date most landscape genetic studies have been conducted with 5–20 neutral, microsatellite markers, in the very near future we will be able to examine hundreds, if not thousands, of regions of the genetic code in hundreds of individuals from across the landscape to make inferences as to the evolutionary forces in play – including natural selection. For example, in evolutionary studies we will be able to disentangle the influences of gene flow and genetic drift from natural selection in influencing the evolutionary trajectory of a population; in the field of conservation biology we will be able to better define Evolutionary Significant Units and Distinct Population Segments, which are the basis for legal protection of species in the United States (Waples 1995); and in the field of ecology we will better predict how climate change will influence continuously distributed populations subject to various selection regimes.

9.1.2 Neutral Versus Non-neutral Molecular Markers

One of the most important differences between the field of population genomics and population genetics lies in the active seeking and utilization of genetic markers under selection – that is areas of the genome that are associated with adaptive traits. Neutral molecular markers, which are not influenced by natural selection, are often used by population geneticists and landscape ecologists because they give unbiased estimates of genetic variation (e.g., heterozygosity), population structure, and gene

flow – the core variables of interest in landscape genetics so far. Used in this context, markers under selection will bias estimates of variation, structure, gene flow, and population relationships. Even a few selected loci among tens of neutral loci can bias estimates of substructure and gene flow metrics such as F_{st} by 10–60% (Luikart et al. 2003; Storz and Nachman 2003) and change the relationships among gene and species trees.

Selection might also change the spatial pattern of relatedness between individuals, which in tern is the basis of most genetic distance measures often used in individual-based approaches in landscape genetics. For example, high levels of gene flow between two study areas should lead to similar allele frequencies in both areas (low F_{ST}), but if certain alleles were eliminated through natural selection (that is the individuals having these alleles died or have reduced fitness) in one of the two study areas, allele frequencies would remain divergent (high F_{ST}) regardless of rates of gene flow. For these reasons geneticists have largely viewed markers under selection as nuisances and thus things to be avoided.

In landscape genomics, however, many markers, both neutral and under selection, will be known for each individual across a landscape. By combining many neutral genetic marker results, allowing us to infer gene flow and drift, with results from markers under selection we could tease apart these evolutionary forces and understand how the landscape and environment are influencing our natural populations. Here the neutral model would serve as the null model (similar to using Euclidian distance as the null model in the wolverine example in Chapter 17) when testing for selection (or among alternative models of selection). Statistical methods to quantify these relationships are currently under development and pose significant challenges (potentially there are thousands of markers with varying degrees of selection, and different modes of selection, e.g. diversifying vs. directional selection). We believe, however, that variants of current methods used in landscape ecology to partial out factors may provide the statistical basis for these analyses. For example, there are well developed methods to associate species distributions with environmental gradients to infer species niche structure as zones of tolerance within an environmental hypervolume (see Chapters 2 and 16, this volume). Conceptually, by replacing spatial occurrence of species with occurrence of particular genetic variants at loci under selection within a particular species it will be possible to apply many of the same conceptual and analytical methods to modeling the patterns of variation in adaptive genes as functions of environmental selection gradients. Specifically, constrained ordination, such as redundancy analysis, is well suited to modeling simultaneous response of multiple genes to complex gradients of multiple environmental variables to identify main factors driving patterns of selection. In addition, the familiar logistic regression is a powerful tool for identifying non-random distributions of single genetic variants as functions of environmental gradients. These approaches will allow the identification of genetic markers potentially under selection by discriminating between those that vary randomly with respect to environmental gradients from those that have strong associations with different environmental conditions. This would suggest potential for differences in selection.

There will be three primary challenges in this effort. First is the challenge of identifying genes under selection from the vast background of genomic data (described further below). The second challenge will be identifying the interaction of multiple genes through epistasis, pleiotropy and gene expression on fitness (Foll and Gaggiotti 2008; Balkenhol et al. 2009). The third main challenge will be identifying the proper environmental variables at the proper spatial and temporal scales that drive the selection processes that result in spatial differentiation in these genes. Ultimately, the challenge is to associate patterns of adaptive genes within organisms with the environmental gradients primarily related to fitness differences of these alternative genetic states (Holderegger and Wagner 2008).

9.1.3 Finding Genes Under Selection

From the discussion of neutral and non-neutral markers above, it is clear that population genomics requires the identification of many neutral and non-neutral markers, and their clear separation. Furthermore, as already noted, confusing neutral markers with those under selection can lead to large errors in interpretation of results. While it is well established how we can obtain neutral molecular markers, the approaches to finding adaptive markers are just now being developed in natural populations. The ideal markers for studying adaptation will be directly involved in the genetic control of adaptive traits, will have a sequence of known function, and will have quantifiable variation (Gonzalez-Marinez et al. 2006). Markers that have these traits will be in or near important functional genes or in gene rich regions. Such markers can include microsatellites (Vasemägi and Primmer 2006, Luikart et al. 2008), but more likely will be Single Nucleotide Polymorphisms (SNPs), AFLPs, and DNA sequences (Box 9.1).

Box 9.1 Molecular Markers for Landscape Genomics

The ideal DNA analysis technology for landscape genomics should genotype hundreds of polymorphic markers (including neutral and adaptive gene markers) that cover the entire genome in a single, simple and reliable experiment. At present AFLPs, SNP multiplex genotyping, and massively parallel sequencing partially or completely fulfill these requirements.

AFLP (Amplified Fragment Length Polymorphism)

AFLP genotyping uses selective PCR to produce hundreds of polymorphic markers that cover the entire genome. However, AFLP markers sometimes cluster around chromosome centromeres. AFLPs have been used to identify

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Box 9.1 (continued)

markers associated with traits that are under selection in non-model plant and vertebrate species (Wilding et al. 2001; Bonin et al. 2006)). Variants of the classical AFLP protocol use one primer that contains a conserved sequence of a gene family (gene-targeted AFLP) or primers in widely-dispersed repeated sequences such as small inserted nuclear elements (SINEs; for example, Alu repeats; van Tienderen et al. 2002). Unlike the classical AFLP protocol, the SINE-based approach requires only a single PCR. Gene-targeted AFLP can facilitate the detection of selection signatures and adaptive genes. Gene targeting (or avoidance) can also be facilitated by using GC-rich (or GC-poor) restriction enzymes, which tend to cut genomic DNA in gene-rich (or gene-poor) regions. The main problems with AFLPs are they are dominant markers (making heterozygote identification difficult), repeatability can be questionable, and transferability between labs is problematic.

SNPs (Single Nucleotide Polymorphisms)

The rapid identification of numerous SNPs (including functional SNPs) is becoming feasible in non-model species (e.g., Vera et al. 2008), owing to the rapid growth of expressed sequence tag (EST) databases, data base mining software, large scale multiplex PCR strategies (Porreca et al., 2007), DNA "capture" techniques (Hodges et al. 2007) and, most importantly, massively parallel sequencing (Shendure and Ji 2008; see below). Recent improvements in SNP genotyping technology (Perkel 2008) make SNPs attractive for population genomics (Kim and Misra 2007). For example, 48 or 96 SNPs can be screened in 96 individuals in a few hours for a cost of only US$0.10–0.20 per SNP (Hauser and Seeb 2009; see also Illumina in online links box). A drawback of SNPs is that they are prone to severe ascertainment bias – bias in estimating genetic diversity and population parameters – which arises when choosing markers on the basis of their polymorphism level, when identifying SNPs using few individuals, or when transferring markers between populations (Morin et al. 2004).

Microsatellites

Microstallites often can be identified in or near genes thanks to genome sequences (Da Silva et al. 2008) and EST data bases (Vasemägi et al. 2005; see also Thurston and Field 2005). However the genotyping of hundreds of microsatellites would require too many DNA amplifications to be competitive with methods that allow a 'massively parallel' analysis (for example, AFLP, SNPs, and next generation sequencing).

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Box 9.1 (continued)

DNA Sequences

We can now generate sequence data for 100s of loci in dozens of individuals from non-model species relatively quickly (Shendure and Ji 2008; Meyer et al. 2008; Vera et al. 2008). Sequence data are desirable because ascertainment bias is reduced (compared to SNPs) or avoided, haplotypes can be identified (or inferred), and coalescent times and allele relatedness (genealogies) can be estimated. Difficulties with sequencing include the analysis of heterozygous sites, homopolymers (consecutive instances of the same base such as AAA), and insertion/deletion polymorphisms; DaSilva et al. 2008). Next-generation sequencing allows generation of hundreds of millions (or billions) of base pairs of sequence in days. Unfortunately, it is still difficult to sequence many individuals. Yet, techniques (such as gaskets), commercial kits and barcoding of primers are being developed to allow simultaneous sequencing of 10 s of loci for approximately 10–200 individuals in an single run on a new generation sequencer (Meyer et al. 2008).

There are a growing number of statistical approaches to detect selection or molecular adaptation. Among the most widely used approach is based on F_{ST}-outlier tests (Beaumont 2005). However this approach assumes discrete populations and alternatives are needed. To detect selection, it will be extremely helpful to have markers in genes with functions related to environmental phenotypic gradients observed across the landscape meeting the criteria listed above. For most wild species we do not have the ability to conduct extensive experimental or captive studies over many generations to find these genes. Thus, we can turn to candidate gene approaches, where we use genes from model or semi-model species with known function and then use simulation modeling to test if these genes appear to be under selection in the wild (Antao et al. 2008). The second approach, often called model free methods, uses population genomic data and examine hundreds of markers with unknown function (Lawson Handley et al. 2007). Here there are several new techniques available to test for non-neutral loci (e.g. Joost et al. 2007), but they fundamentally are usually examining patterns among populations to look for genetic loci that deviate from patterns of neutrality. There are even new bioinformatic programs that are able to process large amount of data, and conduct genome-wide tests to identify markers associated with environmental variables (Joost et al. 2007).

9.1.4 An Example of Landscape Genomics

There have only been a few studies that we would consider landscape genomic studies published thus far. One of the best examples is the use of both neutral genes and genes under selection to understand both the evolution of humans and

the ecology of pathogens (Prugnolle et al. 2005a, b). In the first paper these authors use landscape genetics to provide support for the "recent African origin" model of human evolution by showing that geographic distance from East Africa along probable colonization routes is the best predictor for neutral genetic diversity in human populations. Subsequently, these authors examined patterns in MHC loci (associated with resistance against pathogens; also called Human Leukocyte Antigen or HLA) across 61 human populations to test a hypothesis regarding the high genetic diversity found in MHC loci. Prugnolle et al. (2005b) showed, using landscape resistance models, that the MHC loci had greater variation in areas with high pathogen diversity, while accounting for the fact that the contemporary pattern of diversity at this locus worldwide was influenced by human colonization. While still working with relatively few markers, Prugnolle et al. (2005a, b) demonstrate the power and potential associated with contrasting neutral patterns with those under selection. The pattern of neutral genetic diversity allowed these authors to disentangle the effects of past colonization history from patterns of natural selection on a particular locus with important function. We expect more efforts in the near future on both humans and wildlife that combine both marker types in a fully integrated landscape genomics study.

9.2 Identifying Evolutionary Significant Units Using Genes Under Selection

The field of landscape genomics offers important insights for conservation biologists as well as evolutionary biologists. One fundamental issue in conservation biology is the defining of Evolutionary Significant Units (ESUs), which are population units of conservation interest often below the species level (Fraser and Bernatchez 2001). Defining ESUs has been hotly debated over the past two decades (e.g., Moritz 1994; Waples 1995; Crandall et al. 2000; Fraser and Bernatchez 2001; Palsboll et al. 2007), but the fundamental definition usually suggests that groups of individuals must show adaptive (or ecological) divergence and historical isolation from other groups to be considered ESUs (Allendorf and Luikart 2007).

Historical isolation can be readily analyzed through standard neutral genetic approaches, whereas genetic analysis of adaptive divergence requires the discovery and novel evaluation of genes under selection (see above). Biologists have therefore often looked at historical isolation through molecular genetic studies, but have relied on detailed ecological studies to determine adaptive divergence. Because genetic analysis of isolation can be done quickly and is relatively inexpensive, the designation of ESUs has relied on a plethora of molecular studies and resulting information on contemporary and historical population isolation, but little ecological information. Not surprisingly, ESU designation has been heavily criticized for the overemphasis on reproductive isolation and under-emphasis on ecological data that suggest adaptive differences among populations (Crandall et al. 2000; Pearman 2001).

The heavy reliance on isolation rather than adaptation may bias what we choose to conserve. Consider the species that has multiple populations with high gene flow

but selection for different traits on the landscape of each population. Examining neutral genetic markers across the landscape would reveal high gene flow and suggest for us to lump these populations into one ESU. However, we know that substantial functional divergence and reproductive isolation can take place despite high levels of gene flow (Smith et al. 1997; Crandall et al. 2000; Dieckmann and Doebelli 1999). Here is one place where landscape genomics may help ESU designation. Following the approaches pioneered by Prugnolle et al. (2005a, b) discussed above, we could genetically evaluate both isolation and adaptation, and use the gene flow rates to scale the degree of adaptation present in various sub-populations. Not only would this approach be much more powerful and quantitative than current methods, but it would remove the time and cost differential for obtaining measures of isolation versus adaptation.

9.3 Conclusion

Population genomic approaches can facilitate landscape genetics in three main ways. First, genotyping numerous loci provides high statistical power to quantify gene flow, genetic differentiation (F_{ST}), and diversity. Second, analyzing many loci can help reduce biases when measuring gene flow using methods that require the assumption that loci are neutral (e.g., N_e, F_{ST} and migration rates), because analyzing many loci helps identify and exclude loci that are under selection. Third, the measurement of adaptive genes and detection of locus-specific effects could help detect important selection gradients in the landscape. With these tools we can hopefully move away from delineating ESUs based primarily on isolation and move towards ESUs that will conserve based both isolated populations and adaptive differences across space (see Crandall et al. 2000).

The main barriers to the use of genomics approaches for population monitoring are the current expense and, in some taxa, the lack of availability of numerous markers (including markers in genes). Fortunately costs are decreasing and genomic information is rapidly increasing for most species. As pointed out by Hauser and Seeb (2008), these barriers are decreasing exponentially over time. We are therefore on the cusp of answering long-standing ecological and evolutionary questions in secretive and elusive species, thanks to improved noninvasive sampling of elusive species (see Long et al. 2008 for noninvasive methods to sample carnivores) and new technologies for SNP genotyping and sequencing short DNA fragments (Morin and McCarthy 2007). This includes questions about the genetic basis of local adaptation that can be addressed by using genome-wide scans and population genomic approaches (Luikart et al. 2003) to identify and characterize patterns of adaptive genetic variation. It also includes questions about how landscape features influence gene flow and dispersal in natural populations.

It is exciting time to conduct landscape genetic/genomic studies. The recent boom in genetic technological advances and computational approaches in landscape ecology (i.e. Garroway et al. 2008) and molecular biology will lead to rapid advances changing the relatively new field of landscape genomics.

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