CHAPTER 12

The genetics of migration

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Food, water, and protective cover are basic to the survival of all animals. But the changing seasons can transform a comfortable environment into an unlivable one: the food and water supply can dwindle or disappear, plant cover can vanish, and competition with other animals may increase. Many animals therefore face the problem of occupying a habitat that is suitable for only a portion of the year. Animals that possess the ability to perform movements or migrate are in the favourable position that they can exploit seasonally benign habitats at all times of their annual cycle. In many taxa migration is the rule rather than the exception and the most well-known example here is probably birds. Hence, although we will in this chapter use examples from several taxa, we will mainly focus on bird migration, since the genetic basis of seasonal migration is well known in birds. As outlined in the introductory chapter (Box 1.1), we define seasonal migration as the oriented directional movement of individuals of a population between distinct locations including a return journey (e.g. breeding and wintering grounds). The timescale over which these migratory cycles occur can span hours, days, months, or years, and can even be multigenerational (Chapman et al., Chapter 2). Migration requires remarkable navigation abilities in order to combine inherited information on timing and direction into a spatiotemporal orientation programme that allows the animal to return to the same area on a regular basis (Åkesson et al., Chapter 9). The phenomenon of migration includes a complex suite of behavioural, sensory, morphological, and physiological traits. Key migratory traits are (i) migratory direction and orientation skills using compass reference systems to keep this direction and orient or navigate during the migratory journey (Åkesson et al., Chapter 9); (ii) an endogenous time programme that determines both the onset and termination of migratory behaviour (thus also defining the duration of migration), as well as precise timing of physiological adaptations necessary for a successful migratory journey, such as fuelling (hyperphagia) and moult; and (iii) the intensity of migratory activity, which may vary between different populations and during different migratory seasons (i.e. spring and autumn migration; Lindström et al., Chapter 3). Even when kept in captivity, migratory birds express these traits in a very characteristic behavioural repertoire—so-called *Zugunruhe* or migratory restlessness behaviour (Kramer 1949). The inherited time programme equips birds with information on when to moult and start depositing fat in order to cope with the challenge of the migratory journey, when to start their migratory journey, how long to fly, and when to stop. Migratory restlessness behaviour can easily be quantified in nocturnally migrating birds, as it can clearly be distinguished from other movement behaviours. Migratory restlessness behaviours are in very good accordance with the onset, termination, directional orientation, and intensity of migratory activity in wild conspecifics, and thus their precise quantification under controlled conditions in the lab serves as a good proxy for these migratory traits (Berthold 1996).

Animal Movement Across Scales. Edited by Lars-Anders Hansson and Susanne Åkesson.

12.1 How do we know that migratory traits are innate?

When aiming at understanding the genetic architecture of migratory traits, it is important to choose a study species that exhibits a highly diverse repertoire of migratory phenotypes. Ideally the species' repertoire includes a continuum of migratory distances ranging from long-distance migrants to fully sedentary (non-migratory) populations, as well as populations exhibiting a migratory divide, i.e. neighbouring populations that follow different migratory directions. One of these extremely well-suited 'migratory model organisms' is the blackcap, *Sylvia atricapilla.* Most of our current understanding of the genetics of migration is based on common garden experiments with passerines that allow us to disentangle the genetic and environmental origin of phenotypic differences (reviewed by van Noordwijk et al. 2006). These kinds of experiments include a series of large-scale crossbreeding and selection experiments on blackcaps (e.g. Berthold et al. 1992, Helbig 1996; see Fig. 12.1), and displacement experiments in some other species of passerines (e.g. Perdeck 1958, Thorup et al. 2007). These experiments have suggested a considerable genetic component for several migratory traits, and further showed that these traits (when under strong artificial selection) can drastically change within a few generations.

In addition to experimental approaches, there are also 'unmanipulated' examples in the wild, which clearly show that migratory traits are inherited. The common cuckoo, *Cuculus canorus*, is an excellent example where we know that young birds never interact with their parents, but nevertheless know when they should migrate to sub-Saharan Africa. Another fascinating example is the multigenerational migration cycle of monarch butterflies, *Danaus plexippus* (see Box 12.1 and Fig. 2.6). North American monarchs carry the genetic machinery to migrate, but the programme only gets initiated in migratory populations. This scenario clearly demonstrates that the amazing orientation mechanisms involved in multigenerational migration are not learnt, but must be inherited, since there are at least two non-migratory generations between the migratory ones (Chapman et al., Chapter 2). It further highlights the most likely involvement of interactions between environmental cues and the genetic programme, which can differ between populations or even vary among individuals.

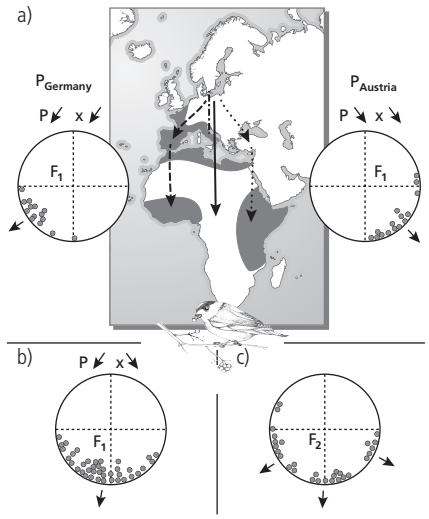

Figure 12.1 Inheritance of blackcap, *Sylvia atricapilla*, migratory direction in a selection- and crossbreeding experiment (modified from Helbig 1996). (a) Orientation diagrams (see also Åkesson et al., Chapter 9) of selectively bred first-generation (F_1) blackcaps during the autumn of parental (P) blackcap populations west (left, dashed) and east (right, dotted) of the central European migratory divide (each dot in the circle indicates the migratory direction of one bird; the orientation vector of all birds as a group is indicated by the arrow outside the circle). (b) F_1 offspring of mixed pairs crossbred in aviaries from parents of either side of the divide show an intermediate orientation phenotype. (c) The scatter of orientation phenotype in the second generation of crossbred offspring (F_2) segregates (compared to F_1) and clusters around both the intermediate and parental phenotypes. This pattern is indicative of a scenario where modulation of the migratory phenotype could be regulated by variation in only a small number of genes with large effects.

12.2 Quantitative genetic assessment of migratory traits

Quantitative genetics (see Box 12.2) allows us to assess the relative roles of genetic variation and environment in explaining the variation of phenotypic traits. For a single trait this relationship, and the response to selection of the trait, is quantified as heritability (h^2) (see Box 12.2 for more information). Migration-related traits that have been investigated

Box 12.1 Genomics of migration: next-generation sequencing approaches

Recent advances in next-generation sequencing (NGS) technology will most likely revolutionize our understanding of the genetics of migration. These resources add a completely new level to the field of migration genetics. This toolbox can now be applied to non-model migratory species and will facilitate the identification of genes or genomic regions that harbour variation with relevant consequences for the migratory phenotype, ranging from the generation of expressed sequence tag (EST) libraries for transcriptional comparison between different migratory phenotypes, up to fully sequenced and annotated genomes of non-model migratory species. However, the basis of migratory phenotype differences might not only lay in the DNA sequence. It is plausible that a limited number of genetic changes can result in large and widespread differences in gene expression downstream in a signalling cascade, either through sequence differences or governed by epigenetic processes. Here we can make use of the fact that migratory animals don't migrate all year round, but the migratory phenotype is only expressed during certain migratory periods. Transcriptomics or gene expression profiling (via RNAseq or microarray technology) allows us to characterize and compare gene expression profiles. This is a promising approach to uncover new gene candidates for further characterization without a priori knowledge of specific genes, and to identify gene networks that are differentially regulated between different phenotypic groups and thus likely involve in modulating the migratory phenotype. Gene expression profiling is typically based on mRNA (transcripts from protein-coding genes) extracts from different tissues (e.g. brain, muscle, fat, liver), and makes it possible to compare gene expression between phenotypically divergent populations at different time points. This approach is expected to be especially powerful in species with populations that exhibit a continuum of migratory phenotypes, i.e. species that include long-distance migrants, short-distance migrants, populations following different migratory directions, or even sedentary populations. The expression of at least some migratory traits is also regulated by external cues, and a lot of phenotypic plasticity could be explained by epigenetic processes. Changes in day length can trigger onset of migratory restlessness, and alterations of the magnetic field have been shown to modulate fuel deposition in birds. The most dramatic example is probably the transition to the migratory phenotype in the desert locust, *Locusta migratoria*, which is triggered by increased serotonin levels owing to social interactions (Anstey et al. 2009). How could epigenetic processes regulate migratory phenotype expression? The migratory cycle of North American monarch butterflies, *Danaus plexippus*, is exceptional in the way that it involves successive generations, including migratory and sedentary populations (reviewed in Brower 1996). All butterfly generations are equipped with the same core genetic material, and it is possible that epigenetic mechanisms are triggering changes in gene expression or function that underlie the migratory phenotype. This means that there is a genetic basis to the migratory process, but the generational divergence in phenotype might be epigenetically regulated by environmental cues that yet remain to be identified.

Another approach to find new potential candidates is to compare the association between sequence polymorphisms and a phenotypic trait. Quantitative trait locus (QTL) mapping requires genetic markers (e.g. single nucleotide polymorphisms (SNPs) or microsatellites) on a linkage map derived from pedigrees of wild populations or from captive populations selectively bred to generate a F_2 or backcross population. Constructing sufficiently large pedigrees (several hundreds to thousands of individuals) of crossbred captive populations is extremely labour- and cost-intensive for many organisms. Genome-wide association studies (GWAS) offer an alternative approach that will be facilitated by NGS technology. GWAS rely on historical recombination events between genetic variants in a population. These events will have a randomizing effect that allows genetic variants to be individually associated with phenotypic traits in a population. This approach can be used in any population but typically requires a large number of individuals and genetic markers.

Using next generation sequencing technology, variation in a large number of phenotypically characterized individuals may be screened to establish associations between genotype and phenotype in non-model organisms. One recent genome-wide association study focused on understanding the genetic basis of migration in two wild populations of migratory steelhead and resident rainbow trout, *Oncorhynchus mykiss*, from the Pacific Northwest (USA). This study identified several genetic loci associated with migratory life-history traits, and suggests a complex multi-genic basis (with several loci of small effect distributed throughout the genome) contributing to migration in this species (Hecht et al. 2013).

Ultimately, we want to assess functionality of any identified candidate gene; i.e. we want to test the effect of the gene on the phenotype expression of the focal trait. To do this it is necessary to modify the genotype (i.e. knockout and rescue mutants). This has, for example, been successfully applied for the monarch butterfly using zinc-finger nucleases, which are a class of synthetic DNA binding proteins that allow targeted genome manipulation (Reppert et al. 2010). However, when working with wild caught migratory animals, generating genetically modified organisms is usually not an option, due to methodological reasons and ethical concerns. In this case the use of small interfering RNA (RNAi)-mediated post-transcriptional gene silencing might be an alternative technological approach.

Box 12.2 Mendelian traits and quantitative traits

Mendelian traits usually have a few qualitative states which are governed by variation at a single locus. The allele can be either dominant or recessive. If the dominant allele is inherited, the dominant phenotype is always expressed—no matter if heterozygous or homozygous for the dominant allele. If the recessive allele is inherited, the recessive phenotype is only expressed if the recessive allele is inherited by the offspring from both the father and the mother, i.e. homozygous recessive. Only a minority of traits are inherited in a purely Mendelian way because dominance may be incomplete or phenotypic traits are controlled by co-dominance (phenotypic expression of both alleles), or variation in traits arise from variation at several loci. Examples of purely Mendelian traits are melanism or the ability to smell hydrogen cyanide (like bitter almond), which are both examples of recessiveness and the immunity to poison ivy, which is an example of dominance.

Quantitative traits are often continuous traits that are influenced by variation at several loci, for example height in humans, wing length in birds, and migration distances across different populations. The environment will often have a large effect on the phenotype. Quantitative traits are commonly measured in terms of their variances. In its simplest form the phenotypic variance can be partitioned into its environmental and genetic component. Of particular interest is the additive genetic variance, which results from the additive effects of alleles at different loci. The ratio between the additive genetic variance and the phenotypic variance within populations is termed *heritability* (h^2). This is a (population) measurement of how much of the phenotypic variance could be explained by the (additive) genetic variance and determines how strongly this trait responds to selection. Note that these measurements are population-specific and are among other things dependent on the environment (e.g. Hoffmann and Merilä 1999, Wilson et al. 2006, Visscher et al. 2008). A constant environment, for example a lab environment, may inflate heritability and, in cases where individuals experience large differences in environmental conditions (e.g. access to food resources), the ordinary heritability of the trait might be underestimated. Since relatives are expected to share a more similar genetic background, heritability could be estimated from comparing a trait among close relatives, for example by linear regression or more sophisticated models such as the animal model, which could take into account more complicated relationships among relatives (Kruuk 2004).

in crossbreeding and selection experiments have generally shown moderate to high heritabilities, which may facilitate rapid evolutionary responses. There is little information on natural variation of migratory traits in wild populations, but in general heritability estimates calculated from quantified behaviour of migratory birds in the lab appear to be similar to heritabilities in the wild (Pulido and Berthold 2003). Values can be estimated by different methods, such as parent–offspring regressions and by full-sibling correlations, or using more sophisticated methods, such as the animal model (Lynch and Walsh 1998, Kruuk 2004). Depending on the modelling approach and the size of pedigree data, estimated heritabilities for migratory activity in blackcaps range from 0.37 to 0.46, and for the timing of (autumn) migration between 0.34 and 0.45 (Berthold and Pulido 1994, Pulido and Berthold 2010). The spread of heritability estimates increases if other species are considered as well (Pulido and Berthold 2003). These quantitative genetic analyses suggest that variation observed within migratory traits has a strong genetic basis. Quantitative genetics also allows us to predict the correlated selective responses of phenotypic traits that are genetically correlated with any focal migratory traits. For example, in blackcaps, genetic correlations have been observed between various timing aspects of migratory restless behaviours such as amount of activity, intensity, and timing (Pulido and Berthold 2003). This suggests that variation in one trait is not necessarily independent of variation of another trait and that traits could be seen as components of a migratory 'gene package' (Berthold 1999). Consequently, selection on a specific trait expressed in the migratory phenotype could ultimately cause indirect selection on genetically correlated traits of the migratory phenotype. Some of these traits might also have consequences on other life-history events throughout the annual cycle. For example, correlations between the timing of migration and moult (Pulido and Coppack 2004), and the timing of migration and breeding (Teplitsky et al. 2011) have been demonstrated.

A quantitative genetics approach is, however, of limited use if we want to understand to what extent the 'migratory gene package' is similar between species across different phylogenetic scales, or to gain insight into the genetic architecture of movement within species. For such comparative analyses, the genes underlying the different components need to be identified. Quantitative genetics analyses have clearly shown that migratory animals have an *inherent time schedule* (Gwinner 1967, 1996; Pulido et al. 2001; O'Malley et. al. 2010; Anderson and Beer 2009; O'Malley and Banks 2008) and at least an inherited initial migratory direction (reviewed in Helbig 1996), which they combine into a spatiotemporal migration programme that leads them to their species- or population-specific winter quarters. We will focus on our current understanding of the genetics of these two key behavioural adaptations.

12.3 The genetics of migratory direction

Small migratory songbirds migrate at night on their own, which means that first-year migrants are heading towards an area they have never been to before. They travel completely by themselves and directional information is thus crucial for their survival (Åkesson et al., Chapter 9). Most of our current understanding of the genetics of directional information comes from crossbreeding and displacement experiments (see Fig. 12.1 for further details and illustration). Crossbreeding experiments of blackcaps from two sides of the central European migratory divide suggest an intermediate mode of inheritance (at least in blackcaps), as first-generation (F_1) offspring of crossbred birds show a directional preference that is intermediate to the parental (P) phenotypes (Fig. 12.1). These experiments also show that genetically determined migratory direction is susceptible to microevolution, i.e. small evolutionary changes that can be detected at the population level. The particular segregation pattern of migratory direction observed in crossbred birds of the second generation (F_2) might suggest a scenario where the modulation of the migratory phenotype is governed by variation in a small number of genes of considerable importance (Fig. 12.1). A different mode of inheritance has been suggested from a study of pied flycatchers, *Ficedula hypoleuca*, and collared flycatchers, *Ficedula albicollis*. Using stable isotope analyses of feathers grown on their wintering grounds as a proxy for wintering location and thus migratory direction, Veen et al. (2007) suggested that the migratory direction of the pied flycatcher, which winters in West Africa, is dominantly expressed in hybrids between the two species. A similar pattern has also been found in the great reed warbler, *Acrocephalus arundinaceus*, and the clamorous warbler, *Acrocephalus stentoreus*, where hybrids appear to follow the migratory route of great reed warblers (Yohannes et al. 2011). Not only birds, but also many fishes perform seasonal mass migrations. A study on Atlantic eels provides indirect evidence for a genetic basis of migratory direction in fish. Both the American *Anguilla rostrata* and the European eel, *Anguilla anguilla*, start their migratory journey in the Sargasso Sea, but their routes differ in distance and direction: American eels migrate towards the North American coast and European eels follow a slightly longer northeasterly route towards Europe, whereas Icelandic eels follow an intermediate migratory direction. A proportion of Icelandic eels could be identified as hybrids based on their genotype, which, again, suggests an intermediate mode of inheritance in migratory direction (Albert et al. 2006). There are only a few studies that have explored the genetics of migratory direction, and too little is known to draw any general conclusions about the mode of inheritance of migratory direction. In general, sex-specific inheritance of migratory traits seems to be negligible, but maternal effects have been found to be significant for the onset of migratory activity in the blackcap (Pulido and Berthold 2010).

12.4 The genetics of migratory timing

In migratory birds, the timing aspects of the migratory route appear to be encoded by the circannual clock of the bird. The circannual clock is a biochemical oscillator that controls the biological rhythm, underlying the timing of behavioural and physiological processes, for example related to breeding, moult, and migration (extensively reviewed

by Gwinner 2003, Wikelski et al. 2008, Visser et al. 2010). As the name suggests this rhythm is only approximately annual and must be synchronized with external cues, of which the most important is day length, in order for the bird to time its behaviours to the natural year. Different components of the endogenous timing programme (i.e. amount, intensity, onset and termination, duration) have been extensively studied in several migratory passerines (reviewed e.g. in Pulido and Berthold 2003). Heritability values for onset of migratory behaviour are medium to high (0.34–0.45) in blackcaps, and even higher estimates have been observed in garden warblers, *Sylvia borin* (0.67 for onset of both autumn and spring migratory activity). Heritability estimates for termination of migratory activity are slightly lower, and in blackcaps they range from 0.16 to 0.44. Values for migratory activity range from 0.36 to 0.52 (for an overview of heritability (h^2) estimates for various traits and species see Pulido and Berthold 2003).

Fish are also well suited for studying spatiotemporal aspects of migration. Recent studies have focused in particular on a genus of trouts and salmons, *Oncorhynchus*, which shows high variability in both spatial orientation and temporal return patterns (Weitkamp 2010). Fish are of great economic value as well, and hatchlings are often tagged with coded wire tags when released from hatcheries. This provides a massive data set on population movement pattern and overall spatial distribution. A candidate gene approach led to the identification of a latitudinal cline in the *CLOCK* gene, a central and molecularly well-characterized component of the circadian clock in animals. *CLOCK* gene variation corresponded with spawning time in the Chinook salmon, *Oncorhynchus tshawytscha*, and identified day length as a primary cue for migrating and spawning in fish (O'Malley and Banks 2008, O'Malley et al. 2010). Genetic data from a pink salmon, *Onchorhynchus gorbuscha*, population further indicate a genetic change for earlier migration timing (Kovach et al. 2012). These findings are in line with a latitudinal cline in *CLOCK* gene variability reported for blue tits, *Cyanistes caeruleus* (Johnsen et al. 2007), which might suggest a general adaptation to ecological factors correlated with latitude, such as breeding or moult phenology (Liedvogel et al. 2009, Caprioli et al. 2012, and Saino et al. 2013 in barn swallows, *Hirundo rustica*). These data suggest that *CLOCK* may be involved in controlling seasonal adaptation and influences geographical variation in timing of reproduction in at least some migratory fish and bird species. However, this picture is far from being consistent, and several studies in fish and birds actually report the lack of significant correlation between the candidate gene *CLOCK* and latitude or the timing of phenological events (Johnsen et al. 2007 (blue throats); Liedvogel and Sheldon 2010; Dor et al. 2012a, b; O'Brien et al. 2013; Peterson et al. 2013). These studies highlight that caution is advised when interpreting variation in candidate genes in the context of seasonal activities, particularly migration, or in relationship to photoperiodism along geographical gradients. One possible explanation may be that this discrepancy in data from various species indicates that this particular 3'UTR polymorphism of the candidate gene is less significant in modulating the expression of *CLOCK* in other species. These studies further highlight how more detailed understanding of the genetic architecture of migratory traits can shed light on other life-history traits, such as the timing of reproduction. One likely consequence of a connection between the timing of migration and breeding might, for example, result in reproductive isolation between sympatric populations, and direct or indirect selection on migratory timing could result in different breeding times (allochrony), reproductive isolation, and eventually speciation.

12.4.1 The threshold model of migration

The results from crossbreeding and selection experiments (Fig. 12.1) not only show that there is considerable genetic variation in migratory traits, but they further suggest that it is possible that many, if not all animals have the genetic machinery to migrate. With appropriate selection pressure, migratory or sedentary behaviour could dominate within a population. This switch between alternate behaviours can occur within very few generations, particularly in populations that include a continuum of migratory strategies, for example in blackcaps (Berthold et al. 1992, Outlaw and Voelker 2006, Rolshausen et al. 2009, Pulido and Berthold 2010).

The evolutionary potential of migratory behaviour is also evident in wild populations. In birds it is quite common to find closely related species that show very different migratory strategies. Furthermore, in species with a wide latitudinal breeding distribution, more northern populations often show a higher frequency of migrants, a reduced sensitivity to environmental cues, and increased migratory distances (Berthold and Querner 1981, Pulido 2011). There are also several instances when a change in migratory behaviour has been recorded over a very short timescale. German blackcaps were first observed to winter in England in the 1960s and the wintering population had increased from 0% to 7% before 1960 and then to 11% in 1992 (Berthold et al. 1992). Similarly, North American house finches, *Carpodacus mexicanus*, have recently expanded their range from the North American east coast, where they were introduced in the 1940s, to most of North America. While the house finch was sedentary over its traditional range in western North America, the newly introduced eastern populations now show migratory behaviour (Able and Belthoff 1998). The mode of inheritance of migratory behaviour has been suggested to be a threshold trait (Pulido et al. 1996; Fig. 12.2). This is a special case of a quantitative trait in which there are two (or several) distinct phenotypic states. The state of an individual depends on a liability variable that is assumed to show a normal distribution in the population. When the combined effect of multiple genes in an individual reaches a threshold, the individual expresses migratory behaviour, and conversely, if the combined effect of the genes is below the threshold, the individual does not express migratory behaviour (reviewed in Pulido 2011; Fig. 12.2: white area under the distribution curve). One important evolutionary consequence of the threshold model is that genetic variation for the alternative state could be hidden from selection in a population. For example, if a population becomes sedentary from an ancestrally migratory state, genetic variation for the expression of migratory behaviour could still be present but be hidden in the resident individuals. Conversely, if selection would favour migratory behaviour in this now sedentary population (for example, due to climate change), this genetic variation could be selected for and the phenotypic trait may subsequently be expressed again (Fig. 12.2, black area under the curve). The threshold model could also be extended to explain a continuum of migratory strategies, such as residency, facultative migration, and obligate migration (Pulido 2011). This could be accommodated by assuming different environmental influences on the threshold. In some populations, such as in obligate long-distance migrants, the threshold might be largely insensitive to environmental cues and whether the individual should migrate is dependent on the genotype. Among more facultative migrants, the genotype might prepare for migration, but the ultimate decision to migrate is also dependent on environmental cues and social interactions (Nilsson et al., Chapter 6). Even though this is a simple and plausible genetic model to explain different migratory strategies, it needs to be verified and explored at a molecular level. Of particular interest is also the interactions between genes and environment that

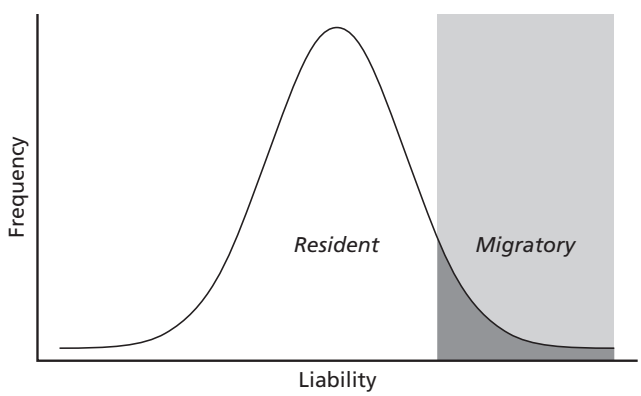

Figure 12.2 The threshold model of migration describes the distribution of migration propensity (i.e. liability) in a partially migratory population comprising both resident and migratory individuals. Individuals with migration propensities below the threshold are residents (white area under the curve); individuals with propensities above the threshold (black area) are migrants. The position of the threshold is not fully fixed and can be shifted to either side by environmental factors (see Pulido 2011 for an in-depth discussion of the model).

could potentially be mediated by epigenetic effects (for an overview on epigenetic consequences on behavioural traits see Champagne 2012, Ledón-Rettig et al. 2012). Epigenetic effects are changes in gene expression that occur without any modification in the genic DNA sequence. Epigenetic factors that govern these expression changes are chemically stable and potentially reversible, and can be modulated or induced by environmental factors and different developmental or physiological stages. Thus, epigenetic effects could, for example, explain some differences between adult migrants and young inexperienced animals on their first migration. In addition, we cannot fully explain how a most likely complex multi-gene adaptation such as migration can repeatedly be lost and gained in the course of evolution until we understand its underlying genetic architecture.

The molecular genetics of the transition from a resident to a migratory phenotype have been explored in trout. In these species of fish, all individuals hatch and are raised in freshwater, but some individuals later undergo a change to a sea-living migratory phenotype through a process termed smoltification (Chapman et al.; Chapter 2). The use of both QTL mapping (Hecht et al. 2012a) and genome-wide association (Hecht et al. 2013; Box 12.1) has discovered the involvement of several genomic regions in the smoltification of the rainbow trout, *Oncorhynchus mykiss*.

12.5 Morphological and behavioural adaptations for migration

Besides behavioural adaptations, the migratory phenotype also includes morphological and physiological adaptations. A well-studied morphological adaptation in migrating animals is wing morphology. Long-distance migrants among birds (Winkler and Leisler 1992) and insects (Lockwood et al. 1998, Alizer and Davis 2010) tend to have longer and more pointed wings than short-distance migrants and sedentary species. A longer wing is beneficial for long-distance flight, but is also thought to decrease manoeuvrability when foraging in dense habitats (Winkler and Leisler 1992; Johansson et al., Chapter 13). A recent scan for genetic variation associated with wing length in great reed warblers, *Acrocephalus arundinaceus*, identified a significant quantitative trait locus (QTL) explaining a substantial part of wing length variability (Tarka et al. 2010). This may be a first important step towards understanding the genetic architecture of wing length and shed light on our understanding of the genetics of associated migratory traits. The genetics of wing morphology has also been studied in the sand cricket, *Gryllus firmus* (reviewed in Roff and Fairbairn 2007). In this species wing length has been characterized as a threshold trait with a strong genetic basis ($h^2 = 0.65$).

Other adaptive traits likely to be genetically controlled are hyperphagia, fat deposition (birds start to eat almost continuously in order to store excess fat to serve as energy during the journey), and organ plasticity (regression of reproductive organs) as preparation for migration, as well as the choice of food, including seasonal shifts from insectivorous to a fruit-biased diet in many migrants before and during the migratory period (e.g. Biebach and Bauchinger 2003, Pulido and Berthold 2003, Piersma et al. 2005).

12.6 Genetics of migration: the molecular toolbox

Many migratory species show a strong phenotypic differentiation between populations (Chapman et al., Chapter 2). Given the data showing that migratory traits have a genetic basis, an obvious question is to ask whether it is possible to link these phenotypic differences to specific genetic differences among populations. A first step is to explore the overall genetic structure between the populations. Several tools have traditionally been used in population genetics to assess genetic structure between populations, for example mitochondrial DNA (mtDNA) or microsatellites, which are often assumed to be selectively neutral and reflect population processes. So far only a few studies have investigated overall genetic structure in migratory species, but the general pattern from various taxa (including birds, fish, and insects) is that intra-specific differences in migratory traits only weakly, or not at all, correlate with the overall genetic differentiation (e.g. Bensch et al. 1999, 2002; Pérez-Tris et al. 2004). This suggests that migratory phenotypes are not associated with

the overall genetic differentiation, but on the other hand, many of the traditional markers only have very limited coverage of the genome. It could also be that variation in migratory traits is mainly modulated by variation at relatively few loci (regions in the genome), and that these subtle but targeted genetic differences may result in dramatic phenotypic effects if they alter the expression levels or up- or downstream signalling cascades of many genes (for example, as transcription complexes). A whole genome AFLP scan in willow warblers, *Phylloscopus trochilus*, a migratory songbird exhibiting a central migratory divide in Scandinavia, lead to the identification of one promising AFLP marker exhibiting a strong genetic differentiation between the subspecies and matches the differences in migratory behaviour across the divide (Bensch et al. 2009; Fig. 12.3). Future characterization and mapping of the chromosomal location of derived AFLP markers on the genome may lead to the identification of the gene(s) under selection. An alternative approach is to limit the study to candidate genes that have shown to have a similar or otherwise interesting function in other species. This approach was used in a study of blackcaps in which longer alleles of a locus with the *ADCYAP1* gene correlated with higher migratory activity (quantified via migratory restlessness activity in night-migratory songbirds) as a proxy for migratory distance (Müller et al. 2011, but see Peterson et al. 2013). As highlighted earlier, results from candidate gene studies must be interpreted with caution and in order to draw general conclusions future studies (e.g. Peterson et al. 2013) are needed to investigate whether this locus is also associated with migratory traits in other birds as well.

For both the AFLP and the candidate gene approach it is further important to bear in mind that genetic variation is not always independently segregating. This non-random association of variation is referred to as linkage disequilibrium and could potentially stretch over large distances of a chromosome. In this case a genetic difference might not be functionally relevant to the trait in question, but is co-inherited and thus strongly correlated with nearby causative functional variation. This highlights that detection of interesting variation in a gene should be accompanied by screening the genetic variation in the nearby chromosome region.

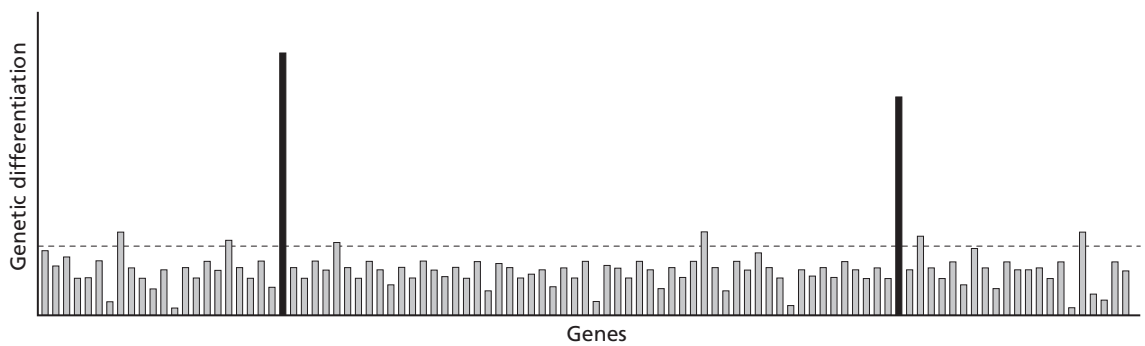

Figure 12.3 A hypothetical example showing genetic structure between two populations exhibiting different migratory phenotypes. The genetic structure is measured as genetic differentiation of several genes. Most genes (grey bars) only show little genetic differentiation between populations around the background level (dotted line). The low differentiation of most genes could be explained by that the populations separated recently and that genetic differences have not had time to accumulate between them, and/or that gene flow between them has homogenized the gene pool. Genes that are under divergent selection between the populations (represented by black bars) are expected to have different variants favoured in each of the two populations and therefore show higher genetic differentiation than other genes. In this particular example, these genes may be involved in adaptations associated with the different migratory phenotypes of each population. However, it is important to keep in mind that the highly differentiated genes could be associated with other adaptations in the populations than those associated with migratory phenotypes. Furthermore, populations are expected to become increasingly genetically differentiated over time due to neutral processes and accumulation of new adaptations. As a consequence, in more genetically separated species, migration genes might be harder to detect because of an overall higher genetic differentiation (dashed line). A suitable species for studying the genetics of migration should thus show very small overall differentiation between migratory phenotypes. In this case, genes involved in migratory phenotypes could be expected to show a more pronounced genetic structure between the populations than is seen with neutral markers.

12.7 Limitations and future perspectives in identifying 'the migratory gene'

Studying the genetics of migration is challenging because migratory traits are complex and often difficult to clearly define, measure, and experimentally manipulate. The complex nature of traits makes it likely that the adaptations may be driven by a large number of genetic variants of which most are expected to have a minor effect (for a general reference see Lynch and Walsh 1998). Behavioural traits have generally been shown to be controlled by genes with small effects (e.g. Flint et al. 2010, Hecht et al. 2013), but genes with major impacts on specific behaviour do exist, as has been successfully demonstrated for gaits in horses and mice (DMRT3, Andersson et al. 2012). Thus, the investigation of genetic variants with large effect size on the migratory phenotype (as expected by the phenotype distribution in crossbreeding experiments with blackcaps, see Fig. 12.1) is of particular interest.

General conclusions about migration genes among taxa is still not possible, because studies of different taxa have frequently focused on different aspects of the migratory phenotype. While some studies have specifically looked at genes involved in e.g. phenological events (*CLOCK* studies in birds and fish), others have focused on genes influencing migration and breeding on a broader scale, such as those responsible for a transition into a migratory phenotype (e.g. Hecht et al. 2012a). Direct comparison is further complicated by the fact that different technological approaches have been used. For example, a study on gene expression patterns may detect genes that are differentially expressed between migrating and breeding individuals. The expression changes of these particular genes that are picked up by gene expression pattern comparison could be due to a downstream effect of an important regulatory sequence variant that may not be detected in this specific data set, but might be detected in a genome-wide association study. In addition, different methods of assessing the same phenotype could potentially explain some differences in gene sets found between different study populations (Hecht et al. 2013). Even in cases where the same candidate gene has been investigated in populations of the same (or a different species), an association established in one of the populations has often been difficult to replicate in another population. This does not rule out the fact that this gene may be of (maybe exclusive) importance in this particular environment, but not in the other populations investigated. However, it is also possible that the same phenotypic effect in other populations could, for example, be produced by different regulatory mechanisms or genetic variants of the same gene, or by other genes in the same pathway. These studies highlight the architecture of complex traits, for which phenotypic variation is likely produced by complex interactions between many genes. Thus taking gene ontology information into account when interpreting these data, such as molecular functions and involvement in a particular biological process of the genes, and attempting to draw general conclusions is extremely important.

The traditional molecular methods that have previously been used in the field of migration research have several limitations. The candidate gene approach is limited by genetic resources available from other populations or species. As highlighted earlier, the same polymorphism in a candidate gene might be of less importance in the new study population, and even if it is important, its particular association with a phenotypic trait should ideally be corroborated with an independent set of genome-wide markers. Similarly, the number of markers is relatively low if one uses microsatellites and AFLPs. This means that only a relatively small fraction of the genome can be screened simultaneously, which clearly limits the resolution achieved. The annotation of these markers also relies on the availability of genomic resources from other closely related species. A general problem of molecular studies of migration has until recently been that none of the model species used as references for genomic analyses of migratory species show a distinct migratory phenotype (e.g. *Drosophila*, zebra finch *Taeniopygia guttata*). Hence, we cannot be sure that the 'migration genes' being looked for are expressed, are functional, or even occur in the model organisms used as default references.

Due to the recent advances in technology, more and more genomic resources of migratory species are becoming available (e.g. monarch butterfly, Zhan et al. 2011; *Ficedula* flycatchers, Ellegren et al.

2012; and willow warbler, Lundberg et al. 2013), which will ultimately start an entirely new era and allow us to validate the function of identified gene candidates. These resources will be invaluable for large-scale analyses, such as high-throughput genotyping and quantification of gene expression or 'next generation sequencing' (NGS; Box 12.1). The detection of migration genes is likely to also be aided by parallel advances in tracking technologies (Åkesson et al., Chapter 9, and Nilsson et al., Chapter 6), which allow for more precise phenotyping of migration in wild populations. These phenotyping improvements will also serve to enhance our understanding of environmental effects on migration and subsequently also genotype versus environment interactions. Once differently expressed gene or variable genomic regions have been identified, it will be possible to test how this variation relates to the phenotypes of individuals, and how epigenetic changes contribute to variation in migratory traits. Consequently, this will lead to an understanding of the genetic architecture of complex traits and ultimately the association between the variation in the environment and the genotype of an individual.

Key questions that could be answered once migration genes have been identified are, for example: Have the same genes been used in migratory traits across distantly related taxa such as insects and birds? How many independent 'genetic solutions' to migratory behaviour have there been? What behavioural adaptations has migration evolved from? What molecular, behavioural, or physiological processes have these groups of genes been involved with in the past before they specialized to shape migratory traits? The future for solving these questions is very exciting and given recent advances, the field of migration genetics certainly looks bright.

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