Crossing Species Boundaries

The Hybrid Histories of the True Geese

Jente Ottenburghs

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ABSTRACT

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Hybridization, the interbreeding of different species, is a common phenomenon in birds: about 16% of bird species is known to have hybridized with at least one other species. Numerous avian hybrid zones have been studied from a morphological or genetic perspective, often documenting the interspecific exchange of genetic material by hybridization and backcrossing (i.e. introgression). The incidence of hybridization varies among bird orders with the Anseriformes (waterfowl: ducks, geese and swans) showing the highest propensity to hybridize. In this thesis, I provide a genomic perspective on the role of hybridization in the evolutionary history of one particular anseriform tribe, the Anserini or "True Geese", which comprises 17 species divided over two genera: *Anser* and *Branta* . The diversification of this bird group took place in the late Pliocene and the early Pleistocene (between four and two million years ago), conceivably driven by a global cooling trend that led to the establishment of a circumpolar tundra belt and the emergence of temperate grasslands. Most species show a steady population increase during this period, followed by population subdivision during the Last Glacial Maximum about 110,000 to 12,000 years ago. The combination of large effective population sizes and occasional range shifts facilitated contact between the diverging goose species, resulting in high levels of interspecific gene flow. Introgressive hybridization might have enabled these goose populations to quickly adapt to changing environments by transferring of advantageous alleles across species boundaries, increasing standing genetic variation or expanding phenotypic variation of certain traits (e.g., beak morphology). Hybridization seems to be a common and integral component in the evolution and diversification of geese. The pervasiveness of rapid speciation and hybridization in geese complicates the attempt to capture their evolutionary history in a phylogenetic tree, therefore I advocate a phylogenetic network approach. Indeed, trying to capture the complex diversification of the True Geese in a branching tree can be regarded as a wild goose chase.

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Chapter 1

General introduction: homage to Saint Martin or why are there still so many kinds of geese?

1.1 General Introduction

The title of this general introduction refers to the classic paper by Hutchinson (1959), *Homage to Santa Rosalia or Why Are There So Many Kinds of Animals?*, in which he developed an ecological theory to explain species diversity and coexistence. The homage to Santa Rosalia refers to the patroness of Palermo, the Italian city where he found inspiration for his theory by observing Water Boatmen (family Corixidae) in a small artificial pond close to a relic for the patroness. The inspiration for this thesis is also based on an observation, namely the occurrence of numerous hybrids between several goose species. How do these goose species coexist and remain distinct despite hybridizing? In other words, why are there *still* so many kinds of geese? In this thesis, the homage refers to Saint Martin of Tours, the patron saint of geese.

Hybridization, the interbreeding of different species, has intrigued the earliest students of natural history. For example, in *Historiae Animalium* Conrad Gesner (1560) reported several peculiar hybrids, such as the Jumar (an alleged cross between a donkey and a cow). Most of the hybrids described by Gesner (1560) turned out to be fictional, but the interest in hybridization did not diminish (Zirkle, 1935). However, opinions about the importance of hybridization in evolution differed: some regarded hybridization as a creative evolutionary force (Lotsy, 1916, Anderson, 1949, Anderson and Stebbins, 1954, Lewontin and Birch, 1966), while others considered it a byproduct of the speciation process (Mayr, 1942, Mayr, 1963, Dobzhansky, 1937, Templeton, 1989). The development of molecular techniques in combination with the study of hybrid zones led to more insights into the dynamics and outcomes of hybridization (Barton and Hewitt, 1985, Moore, 1977). Currently, the significance of hybridization in evolution is acknowledged (Schwenk et al., 2008) as it is involved in several important evolutionary processes.

There is a complex interplay between hybridization and speciation (Abbott et al., 2013, Seehausen, 2004): on the one hand, hybridization can slow down or reverse the speciation process (Seehausen, 2006, Seehausen et al., 2008a), while, on the other hand, it can contribute to the completion of speciation by means of reinforcement (Howard, 1993, Servedio and Noor, 2003) and even give rise to new species by hybrid speciation (Mallet, 2007, Mavarez and Linares, 2008, Schumer et al., 2014). In addition, backcrossing of fertile hybrids can amount to the exchange of genetic material between species, a phenomenon known as introgression (Anderson, 1949, Mallet, 2005). Introgressive hybridization can facilitate the interspecific transfer of adaptive traits (i.e., adaptive introgression; Arnold et al., 2008, Arnold and Martin, 2009, Hedrick, 2013).

Hybridization is mostly rare on a per-individual level, but can be common on a species level (Mallet, 2005). For example, hybrids between European mammals have been recorded for at least 6% of the species (Gray, 1972) and for butterflies the estimates of hybridization incidence range from 6% to 23%, depending on the family (Mallet, 2005). Grant and Grant (1992) calculated that about 10% of bird species have hybridized with at least one other species. The incidence of hybridization varies among bird orders with Anseriformes (waterfowl: ducks, geese and swans) showing the greatest propensity to hybridize. The study of waterfowl hybridization is biased towards ducks, as exemplified by an extensive inventory of hybrid ducks (Gillham and Gillham, 1998), an analysis of hybrid duck fertility patterns (Tubaro and Lijtmaer, 2002) and various genetic studies on introgressive hybridization (Kraus et al., 2012, Lavretsky et al., 2016, Peters et al., 2014a, McCracken and Wilson, 2011, Joseph et al., 2009). The knowledge on goose hybridization is clearly lagging behind.

The True Geese are classified in the waterfowl tribe Anserini (Order Anseriformes, Family Anatidae, Table 1.1) and have been traditionally divided over two genera: *Anser* and *Branta* (Delacour and Mayr, 1945). Hybrids have been reported within each genus (Delnicki, 1974, Hatch and Shortt, 1976, Leafloor et al., 2013, Nijman et al., 2010, Trauger et al., 1971, Weckstein et al., 2002), but also intergeneric hybrids have been documented (Craven and Westemeier, 1979, Nelson, 1952, Prevett and Macinnes, 1973). Whether the occurrence of goose hybrids is a recent phenomenon or a common feature during their evolution remains to be investigated. Several studies have reported evidence for ancient hybridization in particular bird groups (McCormack and Venkatraman, 2013, Lamichhaney et al., 2015, Fuchs et al., 2013, Peters et al., 2007), suggesting that hybridization might be an integral component of avian evolution (Rheindt and Edwards, 2011). Therefore, I will address the following research question: **What is the role of hybridization in the evolutionary history of the True Geese?** A genomic approach will be adopted to answer this question. Genomics has become a standard practise in ornithology (Toews et al., 2016, Kraus and Wink, 2015), opening avenues to answer longstanding questions (Jarvis, 2016).

Table 1.1: Current taxonomy for the True Geese (tribe Anserini).

English Name	Scientific Name	Subspecies
Genus ANSER		
Swan Goose	*Anser cygnoides*	
Taiga Bean Goose	*Anser fabalis*	*A. f. fabalis* *A. f. johanseni* *A. f. middendorffii*
Tundra Bean Goose	*Anser serrirostris*	*A. s. rossicus* *A. s. serrirostris*
Pink-footed Goose	*Anser brachyrhynchus*	
Greater White-fronted Goose	*Anser albifrons*	*A. a. albifrons* (Eurasian) *A. a. flavirostris* (Greenland) *A. a. gambeli* (Western) *A. a. frontalis* (Western) *A. a. elgasi* (Tule)
Lesser White-fronted Goose	*Anser erythropus*	
Greylag Goose	*Anser anser*	*A. a. anser* (European) *A. a. rubrirostris* (Siberian)
Bar-headed Goose	*Anser indicus*	
Emperor Goose	*Anser canagicus*	
Snow Goose	*Anser caerulescens*	*A. c. caerulescens* *A. c. atlantica*
Ross' Goose	*Anser rossii*	
Genus BRANTA		
Brent Goose	*Branta bernicla*	*B. b. bernicla* (Dark-bellied) *B. b. hrota* (Pale-bellied or Atlantic) *B. b. nigricans* (Black) *B. b. orientalis*
Barnacle Goose	*Branta leucopsis*	
Cackling Goose	*Branta hutchinsii*	*B. h. leucopareia* (Aleutian) *B. h. hutchinsii* (Richardson's) *B. h. minima* (Minima) *B. h. taverneri* (Taverner's)
Canada Goose	*Branta canadensis*	*B. c. moffitti* *B. c. maxima* *B. c. occidentalis* *B. c. fulva* *B. c. canadensis* *B. c. interior* *B. c. parvipes*
Hawaiian Goose	*Branta sandvicensis*	
Red-breasted Goose	*Branta ruficollis*	

1.2 Thesis Outline

The main body of this thesis consists of two parts, each comprised of three chapters. Part I (Chapter 3 to 5) deals with hybridization in birds, while Part II (Chapter 6 to 8) focuses on hybridization in the True Geese (Order Anseriformes, Family Anatidae, Tribe Anserini). **Chapter 2** is a stand-alone, introductory chapter establishing a general framework and clarifying the different concepts that will used throughout the thesis. Part I starts with **Chapter 3**, in which I introduce the Avian Hybrids Project, a website gathering the bulk of scientific literature on avian hybridization. In addition, this chapter provides an overview of the occurrence of hybridization across the different bird orders. In **Chapter 4**, I review the current knowledge on avian hybrid zones and I explore distinct patterns of introgression and their underlying mechanisms. In **Chapter 5**, I discuss the consequences of avian hybridization on phylogenetic reconstructions and I advocate the use of phylogenetic networks. With the knowledge on avian hybridization from this first part in mind, I will focus on the True Geese in Part II. In **Chapter 6**, I introduce the True Geese and summarize the current knowledge of hybridization in this bird group. In **Chapter 7**, I develop a phylogenetic framework using genomic data. This framework is the basis for **Chapter 8** where I explore the role of hybridization in the evolutionary history of the True Geese. Finally, in **Chapter 9**, the synthesis, I will combine the insights from the previous chapters to explore the dynamics of species diversification with hybridization.

Chapter 2

Setting the scene: species concepts, speciation and hybridization

2.1. What is a Species?

> *“No term is more difficult to define than ‘species’, and on no point are zoologists more divided than as to what should be understood by this word.”*
>
> *Henry Alleyne Nicholson (1872)*

Before one can have a meaningful discussion, it is important that everyone is talking about the same interpretation of the concepts involved. Many discussions have been futile because the concepts of debate were interpreted differently by different people. Therefore, it is important to clearly define the most important concepts at the start of a discussion. One of the pivotal concepts in this thesis is the species. Before one can study how species originate, interact and interbreed, one needs to answer a seemingly simple question: “What is a species?”

The debate on the definition of a species, commonly known as the species problem (Richards, 2010), has produced an insurmountable quantity of literature and yet there is still no proper definition of a species. Darwin (1859, p.44) nicely formulated the species problem, a description that still holds today: “No one definition has yet satisfied all naturalists; yet every naturalist knows vaguely what he means when he speaks of a species.” In the following sections, I will highlight some of the philosophical and biological issues of the species problem. My goal is not to resolve the species problem, but rather to provide the appropriate background for the remainder of this thesis.

2.1.1 A Philosophical Perspective on the Species Problem

Metaphysics is a traditional branch of philosophy concerned with explaining the fundamental nature of being and the world that encompasses it. Are the things that we observe real or are they just constructs of our brains? This question can also be applied to the nature of species (Kunz, 2012). Most philosophers advocated an essentialist solution to the species problem, a concept that can be traced back to the Greek philosophers Aristotle and Plato. Essentialism is a philosophical view about natural kinds.

It holds that each natural kind can be characterized by a property that is both necessary and sufficient for membership (i.e. the essence of the specific natural kind). The opposite to natural kinds are conventional or artificial kinds, classes of things that are what they are merely by human convention (Sober, 2000). An example of natural kinds are chemical elements. For instance, the element carbon (C) is a natural kind because all carbon atoms are characterized by an atomic number (Z) of 6 (i.e. the number of protons in the nucleus). If you come across a chemical element with atomic number 6, it will be a carbon atom, irrespective of when or where you find it. This reasoning holds for all other chemical elements, which are all characterized by their own, unique atomic number. So, the atomic number is the essence of a chemical element.

The application of essentialism to the species problem resulted in the concept of species essentialism, which Sober (1980) described as follows: "An essentialist view of a given species is committed to there being some property which all and only the members of that species possess." Species essentialism has been advocated by several philosophers (Kitts and Kitts, 1979, Devitt, 2008), but a given species cannot always be characterized by one essential property. This led to the idea that species essences may be a disjunction of a set of properties (based on Wittgenstein, 1958): one individual may have one subset (or cluster) of relevant properties and belong to species A, whereas another individual may have a different subset and also be a member of species A (Boyd, 1999, Wilson, 1999).

There is, however, one main problem with species essentialism: essences (and sets of essential properties) are timeless and eternal, while species are subject to evolutionary change (Brogaard, 2004). It is usually assumed that the idea of species essentialism persisted from Aristotle and Plato through pre-Darwinian naturalists, such as Linnaeus, Buffon and Cuvier, until it was overthrown by the Darwinian revolution. It is often stated that Darwin killed essentialism in biology (e.g., Dennett, 1995, Mayr, 1982). But this so-called "essentialism story" is misleading: many pre-Darwinian naturalists, including Aristotle and Linnaeus, did not adhere to a strict species essentialist philosophy (see Richards, 2010 for a nuanced version of the essentialism story). Regardless of this historical issue, two approaches that try to reconcile the contradiction between species essentialism and evolutionary change are most promising, namely species as historical natural kinds (LaPorte, 2004) and species as "individuals" that change over time (Ghiselin, 1969).

Traditional essentialism treats species essences as intrinsic properties, such as morphological characteristics. Extrinsic or relational properties, on the other hand, require reference to something external. For instance, being a brother is an extrinsic or relational property that depends on something external, in this case another sibling. The idea of species as historical kinds is based on parentage: an organism is a member of a species if its parents are members of that species. Ruse (1987) illustrated this concept nicely with his dog Spencer: "So why do we want to say that he [Spencer] is part of the species? Because he descended from the original ancestors, along with the rest of the group. […] Descent is starting to look very much like an essential property." The members of a species must have relevant relations to their evolutionary ancestors, and this evolutionary history may explain certain features associated with a species, such as morphology and behaviour. So, the essence of a species is its location in the evolutionary tree (LaPorte, 2004, Griffiths, 1999).

Ghiselin (1969) abandoned species essentialism and introduced the concept of species-as-individuals: "An individual occupies a definite position in space and time. It has a beginning and an end. Once it ceases to exist it is gone forever. In a biological context this means that an organism never comes back into existence once it is dead, and a species never comes back into existence once it has become extinct. And although it might move from one place to another, there has to be a continuity across space as well as through time." This view of species as individuals has been advocated by several philosophers and biologists (Hull, 1976, Falk, 1988, Brogaard, 2004).

This philosophical perspective on the species problem thus provides us with two concepts: (1) species as historical natural kinds, and (2) species as individuals. The former concept is popular among philosophers, probably because thinking in terms of natural kinds has a long tradition in philosophy. Biologists, on the other hand, mostly adhere to the species-as-individuals concept, because it is more coherent with evolutionary theory (Richards, 2010). However, both concepts emphasize the importance of (evolutionary) history in the species problem.

2.1.2 A Biological Perspective on the Species Problem

During the 20th century, there has been a proliferation of species concepts. Mayden (1997) lists no less than 22 distinct species concepts, including some widely applied concepts such as the Biological Species Concept (Mayr and Ashlock, 1991), the Phylogenetic Species Concept (Cracraft, 1983) and the Recognition Species Concept (Paterson, 1993). Despite this plethora of species concepts, the so-called silver bullet species concept, one that is universally applicable, has not yet been achieved. The failure to develop a universal species concept can be traced back to two main issues: (1) the pluralistic nature of species and (2) the tension between conceptualization and delimitation (Hey, 2006). First, the proliferation of species concepts is a direct consequence of the diversity of life: different taxonomic groups require different species concepts depending on their particular characteristics (Mayden, 1997). For instance, the Biological Species Concept, which stresses reproductive isolation between members of different species, cannot be applied to asexually reproducing organisms. Second, the issue of species conceptualization is often confused with the issue of species delimitation (Mayden, 1999): concepts are theories or ideas that are general and may or may not be based on empirical observations, while species delimitation requires a prescribed set of repeatable operations that lead to the outcome of whether a certain group of individuals represent a species or not. Hey (2006) summarized this issue nicely: "As scientists we should not confuse our criteria for detecting species with our theoretical understanding of the way species exist. Detection protocols are not concepts."

To resolve these issues, Mayden (1997) proposed a hierarchy of species concepts, with a primary theoretical species concept and several secondary operational species concepts. He argued that only the Evolutionary Species Concept is suitable as primary concept, because it is theoretically robust and generally applicable. This concept states that a species is "an entity composed of organisms which maintains its identity from other such entities through time and over space, and which has its own independent evolutionary fate and historical tendencies" (Wiley and Mayden, 1997). The remaining concepts are secondary, functioning as guidelines that are essential for the study of species in practice (Sites and Marshall, 2004, Wiens and Servedio, 2000). Together, the primary and secondary species concepts form a hierarchical system. Similarly, De Queiroz (1998) reviewed several existing species concepts and argued that all existing species concepts are variants of a single general concept, which he dubbed "the General Lineage Concept." This concept is essentially the Evolutionary Species Concept advocated by Mayden (1997). So, both De Queiroz (1998) and Mayden (1997) reached a similar conclusion, albeit using a different approach (Hey, 2006, Naomi, 2011).

The work of De Queiroz (1998) and Mayden (1997) culminated in a theoretical triumph in the resolution of the species problem, but many practical problems remain. A comparison of different species concepts reveals that they emphasize certain properties, such as reproductive isolation (Mayr and Ashlock, 1991), systems of mate recognition (Paterson, 1993) or monophyly (Cracraft, 1983), that are considered necessary in species delimitation. The application of different species concepts will often lead to the recognition of different species. However, many of the properties emphasized by these species concepts should not be viewed as necessary, but rather contingent upon history and particular circumstances (de Queiroz, 2005). Therefore, a "life history approach" is warranted, in which different species concepts correspond to different stages in the life history of a species (Harrison, 1998).

In summary, the species problem can be partly resolved by theoretical monism (the Evolutionary Species Concept or General Lineage Concept) in combination with practical pluralism, in which different species concepts correspond to different stages in the evolutionary history of a species.

2.2. Speciation

"If you don't know history, you don't know anything. You are a leaf that doesn't know it is part of a tree."

John Michael Crichton

The philosophical perspective on the species problem raised two interesting viewpoints, (1) the essence of a species is its location in the evolutionary tree and (2) a species can be regarded as an individual which occupies a definite position in space and time, with a beginning and an end. From a (theoretical) biological perspective, a species can be described by the Evolutionary Species Concept (Mayden, 1997) and the General Lineage Concept (De Queiroz, 2007), while taking into account the fact that each species has a distinctive life history (Harrison, 1998). Combining the insights from the philosophical and biological perspectives on the species problem, it becomes clear that in order to understand what a species is, one needs to understand what processes and mechanisms underlie the origin and the preservation of a species. In other words, one needs to study the process of speciation.

2.2.1 Speciation Models

The term speciation was first used by Cook (1906) to describe "the origination or multiplication of species by subdivision, usually, if not always, as a result of environmental incidents." Traditionally, speciation models have been classified in a spatial context, namely the well-known Mayrian triumvirate consisting of allopatry, parapatry and sympatry (Bush, 1975, Butlin et al., 2008). In allopatric speciation, the geographic range of a species is split in two or more isolated populations that diverge by natural selection and/or genetic drift. Parapatric speciation concerns the evolution of reproductive isolation between geographically overlapping populations that still exchange genes to a limited extent. Sympatric speciation refers to the situation in which new species originate from a single ancestral population while inhabiting the same geographical region. From a population genetic perspective, allopatric and sympatric speciation are the ends of a continuum of gene flow (m), with parapatric speciation in between (Figure 2.1, Gavrilets, 2004). From this gene flow perspective, the latter two geographic speciation models (parapatry and sympatry) are combined under the heading divergence-with-gene-flow (Pinho and Hey, 2010, Fitzpatrick et al., 2008).

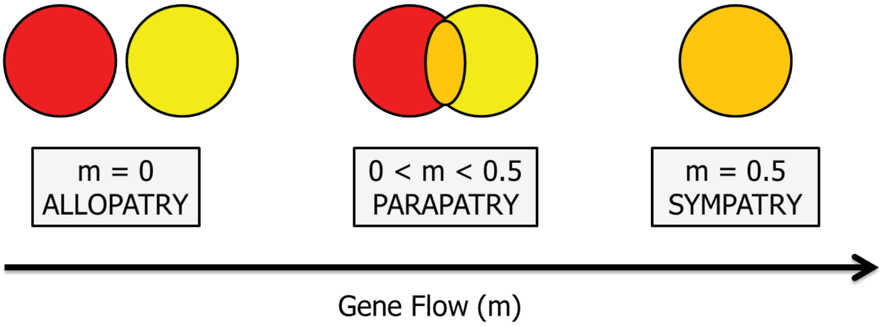


Figure 2.1. Speciation models on the basis of geography and gene flow. Each circle represents the geographical range of a species (in red or yellow). The orange colour indicates that two species overlap in their range. Gene flow is expressed as the migration (m) of alleles or genes from one population to the other and varies between 0 and 0.5. The parapatric and sympatric speciation model are usually combined under the heading divergence-with-gene-flow.

The geographical classification of speciation models has been useful and is still widely applied today (Harrison, 2012). In addition, some more refined geographically-inspired speciation models emerged, such as peripatric (Mayr, 1982), stasipatric (White, 1969), centrifugal (Brown, 1957), microallopatric (Smith, 1965, Paulay, 1985), or allo-sympatric speciation (Mallet, 2005, Coyne and Orr, 2004). However, this geographical classification does have its limitations (Butlin et al., 2008) and other ways to classify speciation models have been proposed (Gavrilets, 2004, Kirkpatrick and Ravigne, 2002, Templeton, 1981).

News ways of classifying speciation may result in a proliferation of speciation models, similar to the situation on species concepts. Indeed, Kirkpatrick and Ravigne (2002) noted that "theoreticians have balkanized the subject of speciation," because each mathematical model focuses on a highly specific scenario. A promising attempt at an overarching "process-based" classification has been made by Dieckmann et al. (2004). They envision speciation as a route through a three-dimensional cube (which I will call the "speciation cube"), of which the axes represent spatial, ecological and mating differentiation (Figure 2.2). At the onset of speciation, there is no differentiation between the populations, which corresponds to the starting point at the origin (i.e. lower left corner). In the classic allopatric model, an external cause (represented by a dotted line) leads to spatial differentiation and the populations consequently diverge under genetic drift (dashed line) or selection (solid line), resulting in mating and/or ecological differentiation.

Sympatric speciation scenarios can also be depicted in this speciation cube. Because no external causes lead to spatial differentiation, the lines are restricted to the front plane of the cube. Divergence can be driven by sexual selection (leading to assortative mating) or ecological differentiation (Bolnick and Fitzpatrick, 2007). An example of differentiation by sexual selection has been documented in Lake Victoria (East Africa), where several sympatric populations of cichlid fish show divergence in male colouration and female preferences (Seehausen et al., 2008b). Differentiation by ecology-based divergent selection is commonly referred to as "ecological speciation" (Rundle and Nosil, 2005, Nosil, 2012). Several examples of ecological speciation involve sympatric phytophagous insect species using different host plants (Berlocher and Feder, 2002), such as the apple maggot (*Rhagoletis pomonella*), which specializes on hawthorns and apples (Feder et al., 1988, Feder et al., 2003).

Finally, speciation cubes can also be used to depict more complex, often multi-phase, speciation processes. For example, Figure 2.2 shows a scenario in which two populations are first geographically isolated and develop partial ecological and mating differentiation in allopatry. Later they re-establish contact and further ecological and mating differentiation occurs. The threespine stickleback (*Gasterosteus aculeatus*) system, in which allopatric marine populations colonize freshwater lakes after glacial retreat and further diverge in sympatry, fits such a scenario (McKinnon and Rundle, 2002).

This process-based approach by Dieckmann et al. (2004) complements the life history approach to the species problem, discussed above (Harrison, 1998). The speciation cube allows for the depiction of many different speciation scenarios, each representing the specific life history of a particular species pair. In all cases, however, there is the build-up of reproductive isolation between two or more populations. In the next section, I will explore the numerous ways in which reproductive isolation can arise.

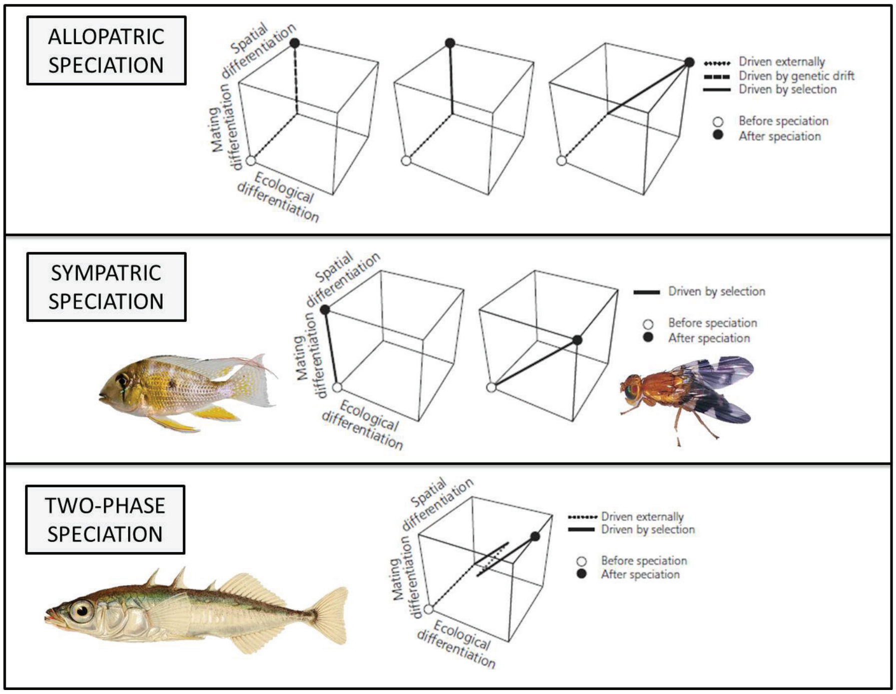


Figure 2.2. A process-based representation of speciation as "speciation cubes." In the cube the axes represent ecological (x), mating (y) and spatial (z) differentiation. Divergence between two populations can be driven by external processes (dotted line), genetic drift (dashed line) or selection (solid line). These speciation cubes can be used to visualize different speciation models (see text for further explanation and examples). – Adapted from Dieckmann et al. (2004).

2.2.2 Reproductive Isolation

The analysis of the species problem showed that, from a theoretical point of view, species can be described as "an entity composed of organisms which maintains its identity from other such entities through time and over space, and which has its own independent evolutionary fate and historical tendencies" (Wiley and Mayden, 1997). Sexually reproducing species maintain their identity from other such entities due to reproductive isolation mechanisms, which prevent homogenising gene flow. Hence, the study of speciation (in sexually reproducing organisms) largely focuses on the evolution of reproductive isolation (Coyne and Orr, 2004). A distinction is made between pre- and postzygotic isolation mechanisms: prezygotic isolation mechanisms act before fertilization, whereas postzygotic isolation mechanisms act after fertilization and can be either intrinsic or extrinsic. Intrinsic postzygotic isolation mechanisms lead to sterility or inviability of the offspring, while extrinsic postzygotic isolation mechanisms encompass lower fitness of the offspring for ecological or behavioural reasons, not developmental defects. In the following sections, I will give an overview of the numerous pre- and postzygotic isolation mechanisms with relevant examples. Finally, I will discuss the interplay of pre- and postzygotic isolation mechanisms in shaping the speciation continuum.

2.2.2.1 Prezygotic Isolation Mechanisms

In general, five mechanisms that can cause prezygotic isolation have been put forward (Coyne and Orr, 2004): (a) habitat isolation, (b) temporal isolation, (c) behavioural isolation, (d) mechanical isolation, and (e) gametic isolation (Figure 2.3).

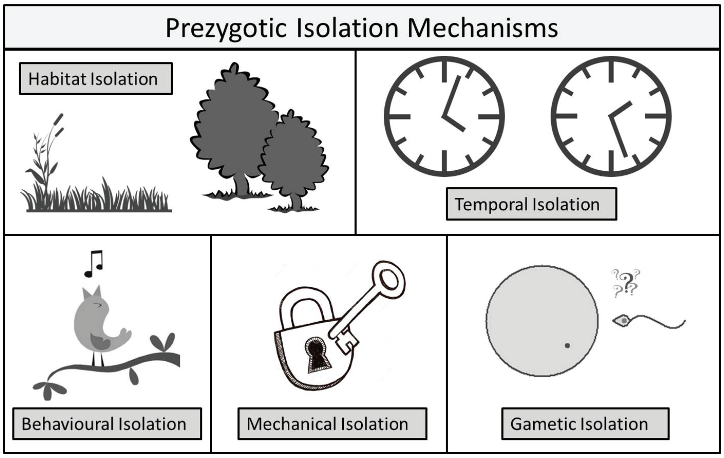


Figure 2.3. An overview of possible prezygotic isolation mechanisms. Detailed descriptions of the different mechanisms can be found in the text.

(a) Habitat Isolation

When populations are spatially separated due to biological differences, leading to a reduction in gene flow between these populations, they show habitat isolation. Although habitat isolation involves spatial separation, it is not identical to geographical isolation. Habitat isolation is a consequence of genetic differences in habitat use between taxa, while geographic isolation is based on historical incidents. In general, habitat isolation entails the inability of a species to use the environment of another species. This can be due to genetically based differences in fitness associated with habitat use, interspecific competition that drives species into different habitats, or the ability of individuals to find habitats to which they are best adapted.

Habitat isolation comes in two forms. In microspatial habitat isolation, members of different species use the same general area, but their reproductive encounters are limited by preferences for or adaptations to different parts of this area. For example, in the North American Red Crossbill (*Loxia curvirostra*) complex nine "call types" have been recognized based on differences in bill morphology. These differences are the result of divergent selection for foraging on different species of conifer (Benkman, 2003). Smith and Benkman (2007) showed that there is reproductive isolation between three types of crossbills in the South Hills (Idaho) due to differences in habitat use, leading to a reduction in gene flow (Parchman et al., 2006). On the macrospatial scale, on the other hand, species cannot interbreed because their habitats are allopatric. However, it is difficult to identify habitat isolation when species are allopatric. The observation that the habitats of allopatric taxa are different does not necessarily indicate habitat isolation (when sympatric these taxa might use the same habitat). Also, even if habitats of allopatric taxa appear similar, the taxa may be divergently adapted to cryptic ecological factors on the microspatial scale. These issues can be tested by transplant or laboratory experiments.

Macrospatial habitat isolation is common in parasites and host-specific insects that are dependent on allopatric hosts. Such habitat isolation can lead to cospeciation, which results in statistically significant congruence between the phylogenies of parasites and hosts (Huelsenbeck et al., 1997). Examples of cospeciation have been described for pocket gophers (Geomyidae) and their chewing lice (Trichodectidae; Hafner and Page, 1995), and sea birds and their chewing lice (Paterson et al., 2000). Similar patterns may arise between plants and their pollinators, such as the intimate relationships between yuccas (Agavaceae) and yucca moths (Prodoxidae; Pellmyr,

2003) and between figs (Moraceae, *Ficus* species) and fig wasps (Agaonidae; Weiblen, 2002). Occasionally, pollination isolation is recognized as a separate prezygotic isolation mechanism (Grant, 1994).

(b) Temporal Isolation

In temporal isolation, gene flow is hampered because members of distinct species breed at different times. The difference in breeding time can range from only a few hours (Montastraea corals; Knowlton et al., 1997) or a couple of months (sympatric forms of the Madeiran Storm Petrel [*Oceanodroma castro*]; Monteiro and Furness, 1998) to several years (periodical cicadas for the genus *Magicicada*; Williams and Simon, 1995). A striking example of temporal isolation has been documented in *Rana* frogs: allopatric populations breed at the same time, whereas the breeding times of sympatric populations are displaced and non-overlapping (Hillis, 1981).

(c) Behavioural Isolation

Behavioural (or ethological) isolation is the lack of sexual attraction between heterospecific individuals (Raychoudhury, 2015). Detecting the existence of behavioural isolation is relatively easy. If closely related species are sympatric and breed at the same time, but do not produce hybrids, one can infer the existence of behavioural isolation. A variety of choice assays (e.g., no-choice, multiple choice) have been applied to measure the strength of behavioural isolation. However, showing which traits are involved is more difficult and requires an experimental approach. For instance, males of the sympatric butterfly species *Pieris occidentalis* and *P. protodice* have different wing patterns: the forewings of *P. occidentalis* are considerably darker. Field observations showed that *P. occidentalis* females only mate with conspecifics and reject nearly all heterospecifics. Wiernasz and Kingsolver (1992) experimentally darkened the wings of *P. protodice* males, which resulted in more interspecific matings between *P. occidentalis* females and *P. protodice* males. This experiment reveals that divergence in wing patterns contributes to behavioural isolation between these butterfly species.

Behavioural isolation is tightly connected to sexual selection (specifically female choice), a link that was laid relatively late (Ringo, 1977, Lande, 1981, West-Eberhard, 1983, but see Haskins and Haskins, 1949). One sex (usually the male) has a trait that stimulates preference in conspecifics but not heterospecifics. The association between a male trait and female preference for that trait can arise and evolve in a variety of ways (reviewed in Kirkpatrick and Ryan, 1991). Moreover, several kinds of traits can stimulate female preference, such as auditory cues (e.g., bird song, Catchpole, 1987) or pheromones (Caro et al., 2015, Steiger and Stokl, 2014).

(d) Mechanical Isolation

Mechanical isolation is the prevention of fertilization between two species due to incompatibilities between their reproductive structures, hampering normal copulation or pollination. These incompatibilities can be the result of divergent morphological features (structural isolation) or because one partner (usually the female) detects abnormal morphology or improper movements of the other partner, leading to termination of the copulation (tactile isolation).

Structural isolation follows the classic 'lock-and-key' model (Shapiro and Porter, 1989), first proposed by Dufour (1844). This concept is best described for the genital morphology of insects (Eberhard, 1985). For instance, the genital morphology among carabid beetles of the subgenus *Ohomopterus* is so different that interspecific copulations result in genital injuries that can lead to mortality of the copulating individuals (Kubota and Sota, 1998, Sota and Kubota, 1998). A classic example of tactile isolation was described for damselflies (Coenagrionidae), in which males fail to grasp heterospecific females with their abdominal appendages and females are able to terminate the copulation (Paulson, 1974).

(e) Gametic Isolation

Reproductive isolation mechanisms that act between spawning or copulation and fertilization lead to gametic isolation (also called postmating prezygotic isolation). There are several stages between insemination and copulation that may serve as barriers to heterospecific sperm (Birkhead and Brillard, 2007, Eady, 2001): the transfer and storage of sperm may fail (Price et al., 2001), the sperm may not be viable in the female reproductive tract (Gregory and Howard, 1994), the cross-attraction between sperm and egg may be lacking (Miller, 1997), or the gametes may be genetically incompatible (Palumbi, 1998, Vacquier, 1998).

The best studied form of gametic isolation is intrinsic gametic incompatibility, which is due to the failure of biochemical recognition mechanisms between heterospecific sperm and egg (Palumbi, 1998, Vacquier, 1998). These mechanisms have been studied extensively in the abalone genus *Haliotus*, in which successful fertilization depends on the complex interaction between the egg protein VERL ("Vitelline Envelope Receptor for Lysin") and the sperm protein lysin (Kresge et al., 2001). Molecular analyses of these proteins revealed signatures of strong selection, indicating rapid evolution (Lee et al., 1995, Swanson and Vacquier, 1998). The rapid evolution of reproductive proteins has also been documented in other animal and plant taxa (Swanson and Vacquier, 2002, Panhuis et al., 2006, Clark et al., 2006, Swanson et al., 2001), suggesting a co-evolutionary arms race between male and female reproductive proteins driven by postcopulatory sexual selection, such as sperm competition and cryptic female choice (Birkhead and Pizzari, 2002). Sperm competition is the competition between sperm of different males to fertilize the egg (Parker, 1970) and cryptic female choice entails the ability of a female to bias the fertilization success of the males inseminating her (Jennions and Petrie, 2000). The rapid evolution of reproductive proteins can also explain the failure of heterospecific sperm to successfully fertilize the egg, because the heterospecific sperm proteins did not coevolve with the female reproductive proteins on the egg that they are attempting to fertilize (Howard, 1999).

2.2.2.2 Postzygotic Isolation Mechanisms

The occurrence of unfit, sterile or inviable hybrids posed one of the biggest problems for Darwinism: why would natural selection allow for the production of unfit offspring? With regard to this problem, Bateson (1922) remarked: "When students of other sciences ask us what is now currently believed about the origin of species we have no clear answers to give. Faith has given place to agnosticism." Attempts to resolve this issue fall into two general classes, which correspond to two forms of postzygotic isolation, namely extrinsic and intrinsic postzygotic isolation.

In the case of extrinsic postzygotic isolation, hybrids may be unfit, because they are not adapted ecologically or behaviourally to the present environment, although they suffer no developmental defects. Their intermediate phenotype needs a non-existent ecological niche that falls between the niches of their parental species (i.e. ecological inviability). For example, the threespine stickleback occurs in two distinct morphs, each adapted to particular feeding conditions: a limnetic morph with a tapered body and a narrow jaw gape and a benthic morph with a broader body and wide jaw gape. Hybrids between both morphs, although perfectly viable and fertile, have an intermediate phenotype, which is not adapted to either of the parental habitats (Hatfield and Schluter, 1999, Rundle, 2002). Hybrids may also show intermediate behaviour that renders them unfit. For example, hybrids between two populations of Blackcaps (*Sylvia atricapilla*) show intermediate migratory behaviour (Helbig, 1991). Birds from one population migrate in a south-western direction, while birds from the other population prefer a south-eastern migration route. The intermediate migration route of the hybrids (straight to the south) sends them to unfavourable wintering grounds (Mettler et al., 2013). Finally, intermediate behaviour may also prevent hybrids from finding a suitable mate (i.e. behavioural sterility). For instance, hybrids between Costa's Hummingbird (*Calypte costae*) and Anna's Hummingbird (*C. anna*) display intermediate courtship behaviour, which fails to attract potential mates (Wells et al., 1978). The examples of ecological inviability and behavioural sterility suggest that extrinsic postzygotic isolation is a byproduct of adaptive evolution (Coyne and Orr, 2004). Indeed, Hatfield and Schluter (1999) concluded that in some cases "the selection pressures responsible for low hybrid fitness in the wild may be responsible for the origin of the species themselves."

This still leaves the issue of intrinsic postzygotic isolation: hybrid sterility and inviability due to developmental defects. Coyne and Orr (2004) highlighted the difficulty to explain intrinsic postzygotic isolation in a Darwinian context: "The problem, then, is to explain how two populations separated by a "fitness valley" can evolve from a common ancestor without either lineage passing through the valley." Attempts to solve this problem involve genetic incompatibilities and chromosomal rearrangements.

The role of genetic incompatibilities (negative epistasis between certain alleles of different genes) in the evolution of postzygotic isolation has been studied extensively (Orr and Turelli, 2001, Orr and Presgraves, 2000). The original mathematical model was formulated by Dobzhansky (1934) and further developed by Muller (1942).

Hence, this model is commonly referred to as the "Dobzhansky-Muller (DM) model." However, Bateson (1909) already published an essentially identical model, apparently unknown to Dobzhansky and Muller (Orr, 1996), to explain the "secret of interracial sterility".

The DM model is intuitively easy to follow: consider two allopatric populations diverging independently, with the same ancestral genotype AABB in both populations. In one population, a mutation (A → a) appears and goes to fixation, resulting in aaBB, which is fertile and viable. In the other population, another mutation (B → b) appears and goes to fixation, resulting in AAbb, which is also fertile and viable. When these populations meet and interbreed, this will result in the genotype AaBb. Alleles a and b have never "met" each other and it is possible that allele a has a deleterious effect that becomes apparent when allele b is present, or vice versa (Figure 2.4). Over evolutionary time, numerous of these incompatibilities may arise, each possibly contributing to hybrid sterility or inviability (Orr, 1995, Gourbiere and Mallet, 2010).

Numerous genetic incompatibilities have been described in *Drosophila* (Presgraves, 2010, Presgraves, 2008), but the most striking example has been documented in hybrids between Swordtail (*Xiphophorus helleri*) and Platyfish (*X. maculatus*). Hybrids between these fish species develop malignant melanomas that are often lethal. The formation of these melanomas is due to overexpression of the *Tu* gene. Normally, the expression of the *Tu* gene is controlled by another gene (R). The Platyfish genome holds both genes that together regulate the formation of black-pigmented dorsolateral spots. In the genome of the Swordtail, however, these genes are not active (resulting in fish which lack black spots). In F_1 hybrids, some individuals inherit the *Tu* gene from their Platyfish parent and an inactive R gene from their Swordtail parent, leading to an overexpression of the *Tu* gene and the development of lethal melanomas (Malitschek et al., 1995, Schartl, 1995). Hybrid lethality in *Xiphophorus* hybrids thus behaves as a simple two-locus DM incompatibility.

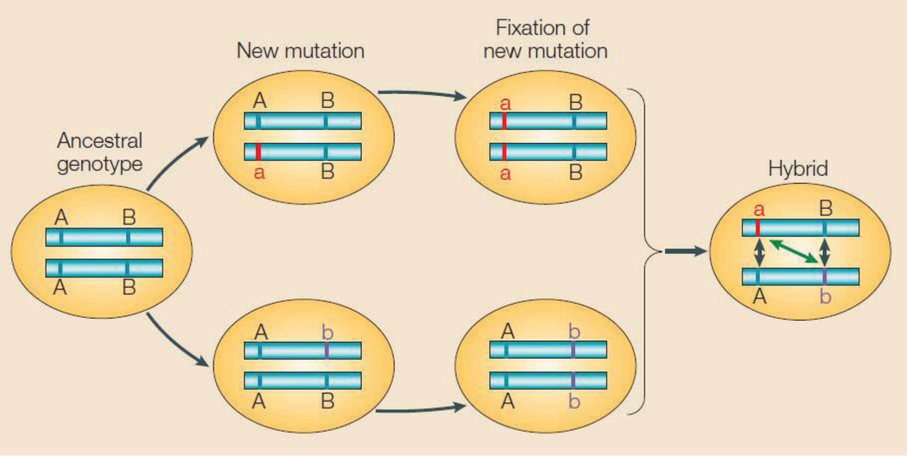


Figure 2.4. The Dobzhansky-Muller model for hybrid incompatibility. Alleles A and B (blue bars) represent the ancestral genotype. Two populations become allopatric: in the upper population a mutation introduces allele a (red bar), while in the lower population a mutation introduces allele b (purple bar). Both alleles go to fixation in their respective populations. When the two populations come into contact, the genotype AaBb might arise. Alleles a and b have never "met" and might thus be incompatible (green arrow), leading to intrinsic postzygotic isolation. – Adapted from Wu & Ting 2004.

Another attempt to explain the occurrence of postzygotic isolation concerns chromosomal rearrangements. Structural changes in chromosomes might directly cause reproductive isolation or physically link genes that cause reproductive isolation (Noor et al., 2001, Rieseberg, 2001, Navarro and Barton, 2003). The first scenario (direct reproductive isolation due to chromosomal rearrangements) is a speciation model known as chromosomal speciation (Faria and Navarro, 2010). The second scenario (physical linkage of genes that cause reproductive isolation) is related to local suppression of recombination rates across the genome.

Chromosomal rearrangements can indeed directly reduce fertility (Greig, 2009). For example, a pericentric inversion (i.e. one that includes a centromere) often causes sterility because this inversion hampers meiosis in hybrids (Rieseberg, 2001). Another type of chromosomal rearrangements causes reproductive isolation in several mammalian species (King, 1995): a centric fusion, also called a Robertsonian fusion or translocation, involves the fusion of two acrocentric chromosomes (i.e. chromosomes with the centromere near the top) into a single metacentric chromosome (i.e.

chromosomes in which the centromere is near the centre). Single centric fusions seem to have small effects on fertility, allowing them to accumulate in populations (Barton, 1980, Baker and Bickham, 1986). When two allopatric populations accumulate different centric fusions and interbreed at a later stage, hybrids would suffer from improper chromosome segregation and consequently sterility. For example, suppose one population experiences a centric fusion between chromosomes 1 and 2, while another population experiences a centric fusion between chromosomes 1 and 3. Because these different fusions both involve chromosome 1, hybrids between these populations will suffer from complications during meiosis (Baker and Bickham, 1986). The fixation of different centric fusions in different populations, leading to so-called chromosomal races, has been documented in several mammalian species, such as mice and shrews (Wojcik et al., 2002, Garagna et al., 2014). In fact, speciation by these centric fusions can be regarded as a chromosomal version of the DM model. Several observational and theoretical issues indicate that chromosomal speciation is relatively rare (Coyne and Orr, 2004).

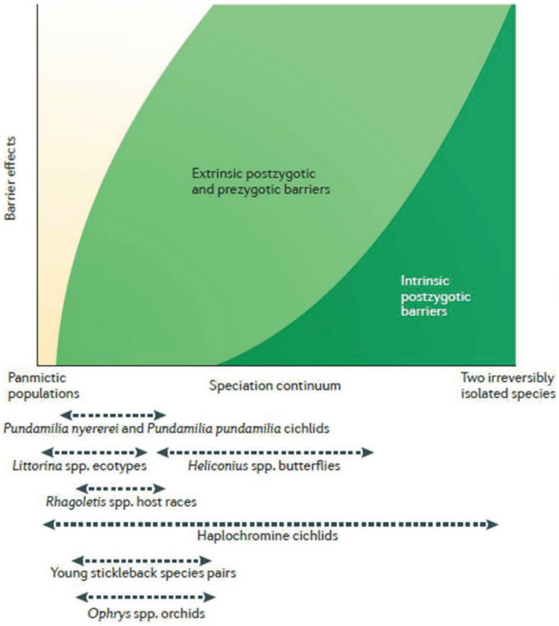
The rarity of chromosomal speciation has shifted the emphasis to the role of recombination in speciation (Ortiz-Barrientos et al., 2002, Butlin, 2005). Rieseberg (2001) argued that "chromosomal rearrangements reduce gene flow more often through their effects on recombination rates than through their effects on fitness." Chromosomal rearrangements can indeed affect recombination rates (Navarro and Barton, 2003). Reduced recombination in a certain genomic region may in turn lead to the physical linkage of genetic incompatibilities or genes involved in reproductive isolation (Noor et al., 2001, Rieseberg, 2001, Felsenstein, 1981). Consequently this genomic region will show reduced gene flow between populations, contributing to reproductive isolation (Noor et al., 2001, Machado et al., 2002, Feder et al., 2003).

In summary, intrinsic postzygotic isolation can be caused by chromosomal rearrangements, genetic incompatibilities or a combination of both. Some of these genetic mechanisms likely play a larger role in postzygotic isolation than others. However, based on the available evidence, it seems that genetic incompatibilities may be the most important cause of intrinsic postzygotic isolation (Coyne and Orr, 2004, Presgraves, 2010).

2.2.2.3 The Speciation Continuum

Reproductive isolation is mostly caused by the combination of several isolation mechanisms. Because these mechanisms often interact, it may be difficult to determine the relative importance of each mechanism. Furthermore, the present importance of a mechanism might be different from its historical importance. However, these issues do not concern us now (for an overview, see Coyne and Orr, 2004, p.55-82). The interplay of different reproductive isolation mechanisms can be depicted as a continuum from a panmictic population to two irreversibly isolated species (Seehausen et al., 2014). Speciation can be driven by divergent sexual or ecological selection, in which case extrinsic postzygotic and prezygotic mechanisms act first and intrinsic postzygotic mechanisms come into play later in the speciation process (Figure 2.5a). Alternatively, speciation can be driven by intrinsic postzygotic mechanisms, such as DM incompatibilities. Extrinsic postzygotic and prezygotic mechanisms accumulate and reinforce reproductive isolation at a later stage (Figure 2.5b). Hendry et al. (2009) recognize four stages across the speciation continuum: (1) continuous variation without reproductive isolation, (2) discontinuous variation with minor reproductive isolation, (3) strong, but reversible, reproductive isolation, and (4) strong and irreversible reproductive isolation. It is important to keep in mind that movement along the speciation continuum is not constant; speciation can go back and forth at different speeds or come to a halt at certain stages (e.g., formation of a stable hybrid zone).

a Speciation driven by divergent selection



b Speciation driven by intrinsic barriers

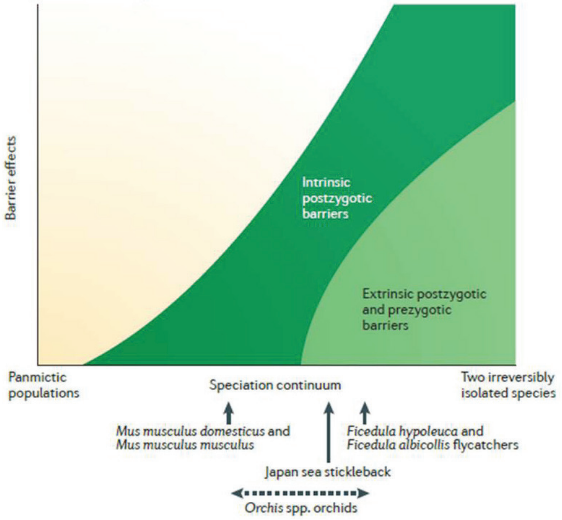


Figure 2.5: The Speciation Continuum of speciation processes driven by (a) divergent selection, where prezygotic and extrinsic postzygotic barriers evolve before intrinsic postzygotic barriers, and (b) by intrinsic barriers, where intrinsic postzygotic barriers evolve before prezygotic and extrinsic postzygotic barriers. The shapes of the curves are hypothetical. The arrows below the graphs indicate the position of several organisms on the Speciation Continuum. – Adapted from Seehausen et al. (2014).

2.2.3 Speciation Genomics

The DM model of intrinsic genetic incompatibilities dominated the study on the genetic basis of speciation. Specifically, the search for genes whose divergence contributes significantly to the evolution of reproductive isolation, so-called "speciation genes", attracted the attention of many evolutionary biologists (Nosil and Schluter, 2011, Presgraves, 2007, Wu and Ting, 2004). The advent of genomic data shifted the focus from individual genes to the whole genome, giving rise to a new field of research: speciation genomics (Seehausen et al., 2014, Nosil and Feder, 2012a).

Genome scans revealed that divergence is heterogeneous across the genome: some regions are more diverged compared to others (Nosil et al., 2009). This observation led to the metaphor of "genomic islands of divergence" (Turner et al., 2005, Harr, 2006, Nadeau et al., 2012), where a genomic island is any region in the genome that exhibits significantly greater differentiation than expected under neutrality. This metaphor draws parallels between genetic divergence across the genome and the topography of oceanic islands, in which the sea level represents the upper limit of expected neutral divergence (Figure 2.6). An island can be composed of directly selected loci and tightly linked (mostly neutral) loci.

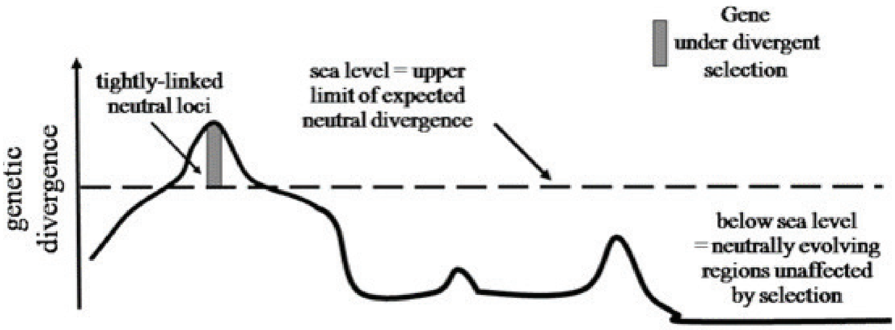


Figure 2.6. Genomic Islands of Divergence. The y-axis represents the degree of genetic divergence across the genome. Genes under divergent selection (gray bar) result in islands above sea level (i.e. upper limit of neutral divergence). Neutral loci that are tightly linked to the gene under selection can increase the size of the islands (left arrow). Genomic regions unaffected by selection will appear below sea level. – Adapted from Michel et al. (2010).

The genomic island metaphor is extensively used in the study of divergence-with-gene-flow to explain how reproductive isolation can built up despite homogenizing gene flow (Nosil and Feder, 2012a). During divergence-with-gene-flow there is an antagonism between selection and recombination: divergent selection creates combinations of locally adapted genes (physically linked in genomic islands), whereas recombination and gene flow break these combinations down (Gavrilets, 2004, Felsenstein, 1981). So, how can genomic islands form and grow despite gene flow? Selection may act on many loci distributed across the genome (i.e. an archipelago of genomic islands) or genomic islands can grow in size when divergently selected loci become physically linked, a process called divergence hitchhiking (Via and West, 2008, Michel et al., 2010, Feder et al., 2012, Feder et al., 2013).

The dynamics of genomic island formation during speciation result in a mosaic genome of neutrally evolving regions and regions under divergent selection (Via and West, 2008). The genomic mosaicism also influences patterns of gene flow, as shown by the studies on hybrid zones (Barton and Hewitt, 1985, Harrison, 1990). A hybrid zone is an area where genetically distinct groups of individuals meet, mate and produce offspring of mixed ancestry (Harrison, 1993). When these hybrid offspring are fertile and interbreed with their parental species, genetic material can be exchanged between the interacting species, a phenomenon known as introgression (Arnold, 2006).

The advent of genomic data indicated considerable variation in introgression rates among individual loci (Payseur, 2010). Alleles can be roughly divided into three categories: (1) neutrally evolving alleles that flow freely between species, (2) alleles that confer an adaptive advantage and introgress quickly, and (3) alleles that compromise hybrid fitness and are not exchanged between species. Hence, most species boundaries are semipermeable: some genomic regions show restricted gene flow while other regions flow freely between the species (Barton and Hewitt, 1985, Barton and Hewitt, 1989). Differential introgression suggests a creative role for hybridization, for instance through the exchange of adaptive alleles between species (Arnold et al., 2008, Hedrick, 2013). In the next section, I will explore the creative role of hybridization in evolution.

2.3. Hybridization

"- The Indominus rex. Our first genetically modified hybrid.

° How did you get two different kinds of dinosaurs to, you know…"

Dialogue from Jurassic World (2015)

The quote at the beginning of this section is taken from the Hollywood blockbuster *Jurassic World*, in which scientists have created a hybrid between different dinosaur species. In real life, scientists have been experimenting with hybrids for a long time (even hybrids between dinosaur species, given that birds are considered the modern descendants of dinosaurs). In the following paragraphs, I will give an overview of the history of hybridization research, before focusing on the creative side of hybridization.

The beginning of hybridization research is largely dominated by botany. At the end of the 17th century botanists developed the theory of plant sexuality. Nehemiah Grew (reprinted in 1965) highlighted the importance of pollen in the reproduction of plants. His work was extended by several researchers, such as Perrault (1688) and Ray (1693). The German botanist and physicist Rudolf Jakob Camerarius (1699) assembled all the evidence for the existence of sexual reproduction in the plant kingdom. Although the theory was widely accepted, by 1700 botanists set out to prove or disprove the new theory. This set the stage for the production of numerous plant hybrids. This period, ranging from 1700 until 1760, is described in detail by Conway Zirkle (1935) in his book *The Beginnings of Plant Hybridization*.

In 1759 the Imperial Academy of Sciences in St. Petersburg announced an open competition "to establish or discredit the sexuality of plants by means of new arguments or experiments apart from those already known, with an introductory historical and physical exposition of all parts of a plant that are believed to contribute something to the fertilization and development of a seed and of a fruit." On July 6, 1760 the prize was awarded to Linnaeus for his entry, entitled *Disquisitio de Sexu Plantarum*. The ideas on plant sexuality also inspired Linnaeus to devise a system of species classification that is still used today.

The German botanist Joseph Gottlieb Kölreuter was sceptical about the crosses documented by Linnaeus and was the first to undertake systematic hybridization experiments. Between 1760 and 1766, Kölreuter published his most important papers: "Vorläufige Nachricht" and three "Fortsetzungen". In these papers, he described numerous experiments that were mainly concerned with unravelling the mechanisms underlying fertilization in plants, such as the nature of pollen and stigmatic secretions (Roberts, 1929). The experiments featured 65 described hybrid crosses involving 13 genera and 54 species. After the work of Kölreuter, more botanists engaged in systematic experiments in plant hybridization. This development was reinforced during the eighteenth and nineteenth century by some of the great scientific academies in Europe that arranged open competitions to solve questions regarding plant sexuality and hybridization (among others, the Physics Section of the Prussian Academy of Sciences in Berlin [1819], the Dutch Academy in Haarlem [1830], and the Paris Academy of Sciences [1861]).

In contrast to the history of plant hybridization research, the records of scientific research in animal hybridization are relatively unknown, although animal hybrids (fantastic or real) were recorded by several naturalists, such as Conrad Gesner (1560) and Georges-Louis Leclerc, Comte de Buffon (1785). The latter held the idea that the male determines the extremities of the body while the female accounts for the internal parts and the overall size and shape. To support his thesis, Buffon described several crosses, including horse x donkey, wolf x mastiff, canary x goldfinch and ewe x goat. He did, however, realize that more evidence was necessary, so he encouraged his readers to engage in more hybridization experiments. Just as the works of Linnaeus and Kölreuter boosted the investigations in plant hybridization, Buffon could have set the experimental work on animal hybrids in motion. But his main failure was the choice of experimental material: Buffon proposed to use horses, wolves and goats instead of fast-breeding animals, such as mice and rats. Using these small rodents conflicted with good taste: "one does not breed with vermin" (Olby, 1966). Consequently, most knowledge on animal hybridization came from the cross-breeding of varieties in order to improve certain breeds. In *The Variation of Animals and Plants under Domestication*, Darwin (1868) discussed several cases of animal hybridization, mostly regarding interbreeding between wild animals and their domesticated counterparts.

The botanically biased knowledge on hybridization certainly contributed to the divergent views on the evolutionary role of hybridization held by zoologists and botanists during the Modern Synthesis, when the union between Mendelian genetics and evolutionary theory was established. Whereas botanists acknowledged the creative force of hybridization in evolution (Anderson and Stebbins, 1954), zoologists remained sceptical (Mayr, 1942, Dobzhansky, 1937). For instance, in *Animal Species and Evolution*, Mayr (1963) stated that "the available evidence contradicts the assumption that hybridization plays a major evolutionary role."

However, the sceptical position of zoologists towards hybridization in animals has changed in the last few decades (Schwenk et al., 2008): the use of new genetic techniques (Lewontin and Birch, 1966) and the study of hybrid zones (Moore, 1977) showed that hybridization and introgression are common phenomena in animal evolution. For example, Dowling and Demarais (1993) noted that "Botanists recognize the importance of introgressive hybridization in evolution. Our results […] indicate that zoologists must do the same." In the following sections, I will highlight some of the creative outcomes of hybridization in animal evolution, namely (1) adaptive introgression, (2) hybrid speciation, and (3) transgressive segregation.

2.3.1 Adaptive Introgression

Alleles and associated phenotypes that cross species boundaries may provide individuals of the recipient species with an adaptive advantage (Arnold, 2006, Hedrick, 2013, Arnold et al., 2008). This phenomenon, adaptive introgression, has been observed in several plant groups, such as the genera *Helianthus* (Whitney et al., 2006, 2010, 2015), *Iris* (Martin et al., 2006), and *Senecio* (Kim et al., 2008). In animals, some putative cases of adaptive introgression have been documented (Hedrick, 2013), including the transfer of mimicry patterns in *Heliconius* butterflies (Dasmahapatra et al., 2012, Pardo-Diaz et al., 2012), rodenticide resistance in mice (Song et al., 2011, Rieseberg, 2011), black coat colour in wolves (Anderson et al., 2009) and beak morphology in Darwin's Finches (Lamichhaney et al., 2015). Recent genome studies revealed genetic admixture between archaic humans, Neanderthals (Green et al., 2010, Vernot and Akey, 2015, Simonti et al., 2016), and Denisovans (Huerta-Sanchez et al., 2014). Some of the introgressed genomic regions show evidence for adaptive introgression (Racimo et al., 2015).

In addition to the interspecific transfer of specific adaptive traits, introgression might also increase the genetic variation in a population (Hedrick, 2013), which is much faster compared to the accumulation of new mutations over time. Anderson (1949) already stated that “raw material brought in by introgression must greatly exceed the new genes produced directly by mutation.”

2.3.2 Hybrid Speciation

Linnaeus was convinced that species were immutable, but his ideas on species immutability were overturned when he received a peculiar specimen of the common toadflax *Linaria vulgaris*, which possessed five spurs instead of one (Gustafsson, 1979). He described it in 1744 as *Peloria* (the Greek word for monster):

> “Nothing can be more wonderful than what has happened to our plant: the deformed offspring of a plant that used to produce flowers of an irregular form have now reverted to a regular form. This is not merely a variation with regard to the maternal genus, but an aberration in terms of the whole class; it provides an example unequalled in the whole of botany, which may now no longer be thought of in terms of the differences between flowers. What has happened is indeed no less wonderful than a cow giving birth to a calf with the head of a wolf.”

Peloria was fertile and produced similar offspring, an observation that convinced Linnaeus that new species could arise. Later, he called upon hybridization as a creative force in the origin of new species: in *Disquisitio de Sexu Plantarum* he states that “it is impossible to doubt that there are new species produced by hybrid generation.” This idea, hybrid speciation, is now defined as “the process in which natural hybridization results in the production of an evolutionary lineage that is at least partially reproductively isolated from both parental lineages and demonstrates a distinct ecological trajectory” (Arnold, 2006).

Two principal types of hybrid speciation are recognized, based on whether or not a change in chromosome number occurs (Mallet, 2007). Hybrid speciation via allopolyploidy (i.e. duplication of chromosomes in hybrids between species) is common in plants (Rieseberg, 1997), but most likely non-existent in animals (Dowling and Secor, 1997). Homoploid hybrid speciation (hereafter HHS) results in a stable, fertile and reproductively isolated hybrid lineage in which the ploidy-level of genome remains the same (Mavarez and Linares, 2008, Mallet, 2007). Schumer et al. (2014) argue that three criteria should be satisfied in order to indisputably demonstrate HHS: (1) reproductive isolation of hybrid lineage from its parental species, (2) genetic or morphological evidence for hybridization, and (3) evidence that reproductive isolation is a direct consequence of past hybridization. Of the many putative cases of HHS among plants (Rieseberg, 1997, Gross and Rieseberg, 2005) and the few among animals (Mavarez and Linares, 2008), only three plant species (Rieseberg et al., 2003) and one species of butterfly (Mavarez et al., 2006) meet all three criteria.

2.3.3 Transgressive Segregation

Richard Goldschmidt (1933, 1940) challenged the gradual nature of Darwinian evolution by invoking macromutations to explain the origin of novel phenotypes. Individuals with such macromutations show phenotypes that lie outside of the range of normal phenotypes, enabling these individuals to fill a novel niche if this is vacant. This model of "Hopeful Monsters" was criticized early on because it was too improbable "to overtax one's credulity" (Dobzhansky, 1937). Recent work on hybrid speciation might revive the concept of Hopeful Monsters (Dittrich-Reed and Fitzpatrick, 2013, Mallet, 2007). Hybridization can result in phenotypes outside the normal range of variation, a phenomenon called transgressive segregation (Stelkens and Seehausen, 2009, Rieseberg et al., 1999).

2.4. Conclusion

The philosophical perspective on the species problem brought forth two interesting viewpoints, (1) the essence of a species is its location in the evolutionary tree and (2) a species can be regarded as an individual which occupies a definite position in space and time, with a beginning and an end. From a (theoretical) biological perspective, a species can be described by the Evolutionary Species Concept (Mayden, 1997) and the General Lineage Concept (De Queiroz, 2007), while taking into account that each species has a distinctive life history (Harrison, 1998). Combining the insights from the philosophical and biological perspectives on the species problem, it becomes clear that in order to understand what a species is, one needs to study the process of speciation.

The study of speciation largely focuses on the evolution of reproductive isolation (Coyne and Orr, 2004). A distinction is made between pre- and postzygotic isolation mechanisms: prezygotic isolation mechanisms act before fertilization, whereas postzygotic isolation mechanisms act after fertilization and can be either intrinsic or extrinsic. Intrinsic postzygotic isolation mechanisms lead to sterility or inviability of the offspring, while extrinsic postzygotic isolation mechanisms encompass lower fitness of the offspring for ecological or behavioural reasons, not developmental defects. Reproductive isolation is mostly caused by the combination of several isolation mechanisms.

The interplay of different reproductive isolation mechanisms can be depicted as a continuum from a panmictic population to two irreversibly isolated species (Seehausen et al., 2014). Speciation can be driven by divergent sexual or ecological selection, in which case extrinsic postzygotic and prezygotic mechanisms act first and intrinsic postzygotic mechanisms come into play later in the speciation process. Alternatively, speciation can be driven by intrinsic postzygotic mechanisms, such as DM incompatibilities. Extrinsic postzygotic and prezygotic mechanisms accumulate and reinforce reproductive isolation at a later stage. It is important to keep in mind that movement along this speciation continuum is not constant; speciation can go back and forth at different speeds or come to a halt at certain stages (e.g., formation of a stable hybrid zone).

Incomplete reproductive isolation can lead to the formation of hybrids. The views on the evolutionary importance of hybridization have changed over time. Recently, it has become clear that hybridization and introgression are common phenomena in animal evolution. Moreover, hybridization can play a creative role in evolution through several processes, such as adaptive introgression, hybrid speciation, and transgressive segregation. The complex interplay between hybridization and speciation will shape the evolutionary history of different populations in different ways, thereby rendering the formulation of a universally applicable species concept nearly impossible. Hence, it is advisable to abandon the philosophical swamp of species concepts and focus on the processes that are responsible for the present-day species diversity.

PART I

AVIAN HYBRIDIZATION

Chapter 3

The avian hybrids project: gathering the scientific literature on avian hybridization

Ottenburghs, J., Ydenberg, R.C., van Hooft, P., van Wieren, S.E., & Prins, H.H.T. (2015). The Avian Hybrids Project: gathering the scientific literature on avian hybridization. *Ibis*, 157 (4), 892-894

3.1 The Avian Hybrids Project

Hybridization, the interbreeding of different species, plays an important role in several evolutionary processes, such as adaptive trait transfer (Arnold, 2006, Arnold et al., 2008, Hedrick, 2013), adaptive radiations (Seehausen, 2004), and the origin of new species (Abbott et al., 2013, Schumer et al., 2014, Mavarez and Linares, 2008). However, hybridization can have detrimental effects for the species involved. One of the species can be driven to extinction (Rhymer and Simberloff, 1996) or two species can merge into one leading to a loss in biodiversity (Seehausen, 2006, Seehausen et al., 2008a). That is why hybridization has also become a relevant topic in conservation (Allendorf et al., 2001, Brumfield, 2010).

The occurrence of hybridization is mostly rare on an individual basis, but can be common on a species level (Mallet, 2005). It was estimated that about 25% of the vascular plants in the United Kingdom hybridize (Stace, 1975) and many plant taxa are probably of hybrid origin (Rieseberg, 1997). In the animal kingdom, hybridization is also a common phenomenon (on a species level), as shown by the number of hybrids documented in different taxonomic groups, such as mammals (Gray, 1972), fish (Hubbs, 1955) and reptiles (Jancuchova-Laskova et al., 2015).

Birds show relatively high levels of hybridization and several estimates of the incidence of hybridization in this taxonomic group have been published (Mayr and Short, 1970, Meise, 1975, Grant and Grant, 1992, Panov, 1989). The most recent is from Grant and Grant (1992), who calculated that 9.2% of all bird species hybridize with at least one other bird species. Since then the occurrence of avian hybrids has been thoroughly researched and more cases have been documented (McCarthy, 2006). We used the IOC World Bird List (Gil and Donsker, 2013) and records, retrieved from the Serge Dumont Bird Hybrids Database (Dumont, 2014), to update the analysis of Grant and Grant (1992). Hybrids between subspecies were not included and a distinction was made between hybridization in nature and in captivity.

Figure 3.1 gives an overview of the incidence of hybridization in all bird orders. All in all, 1714 out of 10446 bird species (16.4%) have been documented to have hybridized with at least one other bird species in nature. Including hybridization in captivity, this figure increases to 2204 species (21.1%). These numbers are most probably underestimates given the general ignorance of breeding biology of several bird groups, such as cryptic tropical species, and the difference in detection probability of particular hybrids (Randler, 2004). Hybridization occurs in the majority of 39 bird orders, with the exception of nine species-poor orders.

The documentation of numerous avian hybrids (McCarthy, 2006) and hybrid zones (Price, 2008) has stimulated the curiosity of many ornithologists and has led to an enormous amount of scientific papers. On the website of the Avian Hybrids Project (https://avianhybrids.wordpress.com/), we gather the bulk of scientific literature on avian hybridization, arranged by bird order (and on a family level for the Passeriformes). Currently, all bird order summaries have been entirely written by the first author, but we encourage experts on certain bird groups to critically review these texts and provide revised versions. In addition, we motivate ornithologists to send us non-technical summaries of their latest papers, which will be featured on the "Latest News" section of the website. The goal of this website is to provide a common place where the current state of knowledge on avian hybridization is presented. We believe that this will benefit the scientific community working on birds in general and avian hybridization in particular. Moreover, this website may act as the birthplace for many fruitful collaborations.

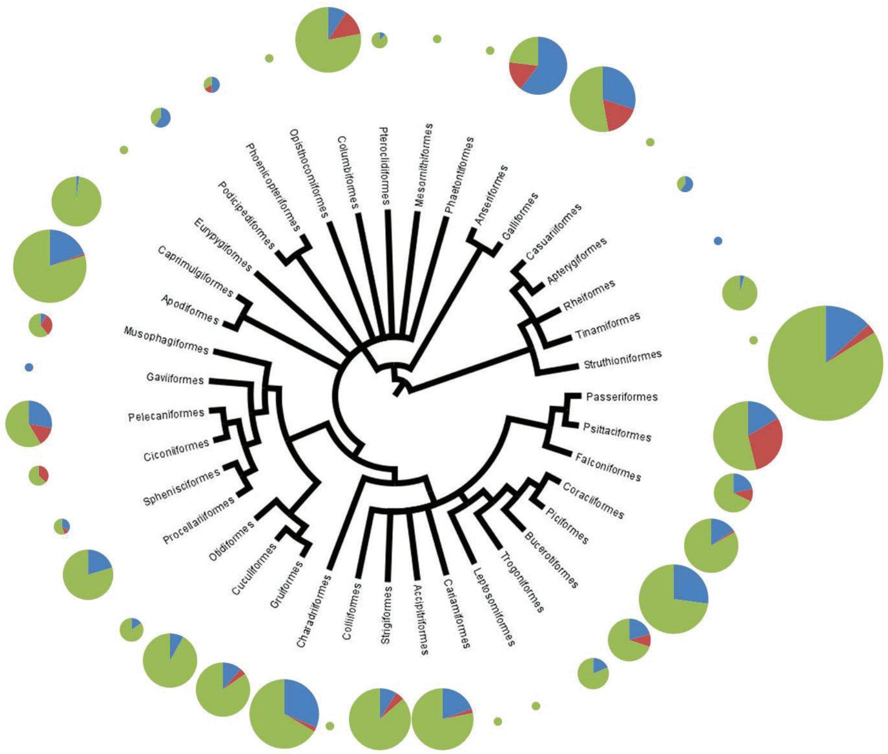


Figure 3.1. The incidence of hybridization in all 39 bird Orders. The size of the pie charts is proportional to the number of species in the respective Order. Colours indicate no hybridization (green), hybridization in nature (blue) and hybridization in captivity (red). Species that hybridized both in nature and in captivity are included only in the former category. The central phylogenetic tree is based on the Tree of Life Project (Maddison & Schulz 2007).

Chapter 4

An overview of avian hybrid zones and patterns of introgression

Ottenburghs, J., Kraus, R.H.S, van Hooft, P., van Wieren, S.E., Ydenberg, R.C. & Prins, H.H.T. (2016). An Overview of Avian Hybrid Zones and Patterns of Introgression. **Submitted**

Abstract

There is an extensive amount of scientific literature on avian hybridization. In this review, we synthesize this knowledge on avian hybrid zones and patterns of introgression. We identified 114 avian hybrid zones that have been described from a morphological or genetic perspective. Most avian hybrid zones have been classified as tension zones, assuming a balance between dispersal into the zone and decreased fitness of the hybrids. Some avian hybrid zones seem to fit the bounded hybrid superiority zone model, in which hybrids are more fit compared to the parental taxa in restricted areas. Moreover, it may be possible that a hybrid zone alternates between tension zone and hybrid superiority zone dynamics. Most hybrids zones are probably the outcome of secondary contact after an allopatric phase, but discriminating between primary and secondary contact zones is challenging. New techniques, such as Approximate Bayesian Computation (ABC) modelling and patterns of genomic divergence, may be fruitful approaches to tackle this challenge. Second, we discuss several striking patterns of introgression. Differential introgression patterns among several genomic classes, such as autosomal, mitochondrial and sex-linked loci, can be explained by Haldane's Rule and sex-biased dispersal. We explore the generality of these proposed mechanisms. Although most introgression patterns are in line with the predictions of Haldane's rule and/or the sex-biased dispersal hypothesis, additional evidence is needed to confidently identify the underlying mechanism. We also consider asymmetric introgression, which can be the result of a numerous processes, ranging from simple demographic processes (e.g., range expansion) to complex behaviours, including interspecific forced copulations and brood amalgamation. Finally, we discuss human-mediated introgression, caused by habitat modification and introduction of non-native species.

4.1. Introduction

Hybridization concerns "the interbreeding of individuals from two populations, or groups of populations, which are distinguishable on the basis of one or more heritable characters" (Harrison, 1990). This phenomenon has intrigued the earliest students of natural history (Zirkle, 1935). The Greek philosopher Aristotle extensively described hybrids in his *Historia Animalium*, where he noted that "natural intercourse takes place between animals of the same kind. However, those also unite whose nature is near akin and whose form is not very different, if their size is much the same and if periods of gestation are equal." He even called upon hybridization to account for the species richness in Africa:

> "And the proverb about Libya [Africa], that "Libya is always producing something new," is said to have originated from animals of different species uniting with one another in that country, for it is said that because of the want of water all must meet at the few places where springs are to be found, and that even different kinds unite in consequence. […] It would appear that in that country animals of diverse species meet, on account of the rainless climate, at the watering-places, and there pair together; and such pairs will often breed if they be nearly of the same size and have periods of gestation of the same length."

Over the period of two millennia, the fascination for hybridization in the animal kingdom had declined. In *Animal Species and Evolution* (1963), Ernst Mayr wrote that "successful hybridization is indeed a rare phenomenon among animals" and stated that "available evidence contradicts the assumption that hybridization plays a major evolutionary role." These statements derived from the fact that animal hybrids are quite often found to be sterile, or suffer from fitness reductions.

However, the sceptical position of zoologists towards hybridization in animals has changed dramatically (reverted to Aristotle, as it were) in the last few decades. A paper that can be regarded as a turning-point in this respect is "Hybridization as a Source of Variation for Adaptation to New Environments" by Richard Lewontin and Louis Charles Birch (1966). They showed that the exchange of genetic material between the fruit flies *Drosophila neohumeralis* and *D. tryoni* could provide genetic variability for adaptation to an extreme environment. The development of new molecular techniques in the following years enabled zoologists to gain more insights into the dynamics and outcomes of hybridization. In the 1970s and 1980s, the combination of molecular markers and the study of hybrid zones (Moore, 1977, Barton and Hewitt, 1989, Barton and Hewitt, 1985) highlighted the role of hybridization in animal evolution. Hybrid zones were also considered "windows on the evolutionary process" (Harrison, 1990) and "natural laboratories" (Barton and Hewitt, 1989, Hewitt, 1988) as a means to study the process of speciation.

Currently, the significance of hybridization in animal evolution is acknowledged (Schwenk et al., 2008). Hybridization plays an important role in several evolutionary processes, such as the acquirement/evolution of new traits via adaptive trait transfer (Arnold, 2006, Arnold et al., 2008, Hedrick, 2013), adaptive radiations (Seehausen, 2004), and the origin of new species (Abbott et al., 2013, Schumer et al., 2014, Mavarez and Linares, 2008). Hybridization can also have detrimental effects for the species involved: one of the species can be driven to extinction while the other remains stable (Rhymer and Simberloff, 1996) or two species can merge into a distinctly new one (Seehausen, 2006, Seehausen et al., 2008a). That is why hybridization has recently been recognized as an important topic in conservation (Allendorf et al., 2001, Brumfield, 2010).

Although rare on an individual level, hybridization can be relatively common on a species level. In butterflies, for example, estimates of hybridization incidence range from 6% to 23%, depending on the family (Mallet, 2005), and hybrids between European mammals have been recorded for at least 6% of the species (Gray, 1972). In birds, about 16% of all species are known to have hybridized with at least one other species in nature, including captive hybridization this figure increases to 21% (Chapter 3). The high incidence of avian hybridization and the ease with which birds can be studied in nature has led to a large output of scientific papers on this subject. In this review, we focus on two aspects of avian hybridization research, namely (1) hybrid zones and (2) patterns of introgression.

There are two excellent overviews on these topics, namely chapter 15 on "Hybrid Zones" in the book "Speciation in Birds" by Price (2008) and the paper "Genetic Introgression: An Integral but Neglected Component of Speciation in Birds" by

Rheindt and Edwards (2011). The goal of this review is to go beyond these two overviews by including new findings and focusing on issues that these overviews did not consider, such as discriminating between primary versus secondary hybrid zones and mechanisms leading to asymmetric introgression. In addition, this review combines important insights on avian hybrid zones and patterns of introgression in one place, thereby providing a modern synthesis of these topics.

4.2. Avian Hybrid Zones

Arnold (1997) defines a hybrid zone as a situation in nature where "two populations of individuals that are distinguishable on the basis of one or more heritable characters overlap spatially and temporally and cross to form viable and at least partially fertile offspring." This definition is very broad and encompasses hybrid zones between species, subspecies, (chromosomal) races and colour morphs. In accordance with Price (2008), we decided to focus on hybrid zones between populations that are at least recognized as subspecies. We used the hybrid zones listed by Price (2008) and the contact zones listed by Haffer (1992) and Ford (1987) as a starting point to assemble an overview of avian hybrid zones (Table 4.1). Every hybrid zone mentioned was checked in Thomson Reuters' Web of Science™ and Elsevier Scopus® for supporting literature; we decided to only list hybrid zones that have been studied in detail from a genetic or morphological perspective. In addition, we searched the same databases for additional hybrid zones using the keywords "hybrid zone*", the results were consequently checked for studies on avian hybrid zones.

Fifty-two hybrid zones listed by Ford (1987), Haffer (1992) and Price (2008) were supported by literature. Our additional literature search uncovered another 62 avian hybrid zones. So, we identified 114 avian hybrid zones of which 85 (75%) have been confirmed using genetic data. The best studied hybrid zones can be found in North America and Europe (Beheregaray, 2008). Europe houses some classic examples of avian hybrid zones, such as the hybrid zone between western Carrion Crow (*Corvus corone*) and Hooded Crow (*Corvus cornix*) that runs from Scotland through Central Europe into Italy (Meise, 1928), and the hybrid zones between Collared Flycatcher (*Ficedula albicollis*) and Pied Flycatcher (*Ficedula hypoleuca*) on the Swedish islands of Öland and Gotland (Alatalo et al., 1982). In North America, the study of the hybrid zones was pioneered by Charles Sibley and his students (Sibley, 1954, Sibley and Short, 1959, Sibley and Short, 1964, Sibley and West, 1959). Some of these hybrid zones have recently been confirmed by rigorous morphological and molecular analyses (Curry and Patten, 2014, Carling et al., 2011, Carling and Zuckerberg, 2011, Mettler and Spellman, 2009). In Australia, a similar scenario unfolded. During the late 1970s and the 1980s, Julian Ford filled the pages of the Australian journal *Emu* with detailed descriptions of numerous contact zones (reviewed in Ford, 1987). Many of these contact zones have recently been studied with the newest molecular tools (Joseph et al., 2011, Kearns et al., 2009, Lee and Edwards, 2008). In South America, Jürgen Haffer (1997) listed a number of putative hybrid zones, but in contrast to North America and Australia, these cases have not been re-examined from a genetic perspective (but see Weir et al., 2015). Similarly, several hybrid zones in Africa and Asia are in need of genetic confirmation.

4.2.1 Hybrid Zone Analysis

Most hybrid zones have been characterized by mapping the geographical distribution of phenotypes, using a hybrid index (an individual is given a value of 0 if it resembles one species and a value of 1 if it resembles another species, with intermediate values for intermediate phenotypes). Fitting a curve through these indices measures the extent of overlap between the two taxa or the width of the hybrid zone (mostly defined as the inverse tangent to the steepest part of this curve). More sophisticated genetic models have been developed to determine the width of a hybrid zone (Barton and Hewitt, 1985). The simplest model assumes a balance between dispersal into the zone and decreased fitness of the hybrids. A hybrid zone that conforms to this model is called a tension zone (Key, 1968). By determining cline width and cline shape across the hybrid zone, it is possible to estimate other parameters, such as dispersal distance, selection pressure and patterns of linkage disequilibrium (Barton and Gale, 1993). For example, the width of a hybrid zone is proportional to the ratio between dispersal distance (σ) and the root of selection (s). Hence, a wide hybrid zone can be the result of high dispersal distances or high hybrid fitness.

Price (2008) reported a negative correlation between hybrid zone width and the age of the hybridizing taxa. Hybrid zones between distantly related taxa are often very narrow and fit the classic concept of a tension zone, namely a balance between dispersal and low hybrid fitness. The reduction in hybrid fitness is mostly caused by intrinsic factors (i.e. independent of the environment in which hybrids occur). For instance, Thrush Nightingale (*Luscinia luscinia*) and Common Nightingale (*L. megarhynchos*) diverged approximately 2 million years ago and form a narrow hybrid zone in Central Europe (Storchova et al., 2010). Captive breeding experiments have shown that hybrid females are sterile due to intrinsic genetic incompatibilities (Stadie, 1991). Hybrid zones between closely related taxa, on the other hand, are often relatively wide and there does not seem to be strong selection against hybrids. For example, the hybrid zone between two closely related gull species, Glaucous-Winged Gull (*Larus glaucescens*) and Western Gull (*L. occidentalis*), is about 800 km wide (Gay et al., 2008) and hybrids have similar (and sometimes even higher) breeding success compared to their parental species (Good et al., 2000). In addition, several wide hybrid zones seem to be associated with the recent spread of one taxon into the range of another (often as a result of anthropogenic change). Theory predicts that, if hybrid fitness is low and the taxa hybridize extensively, these hybrid zones will be temporary and result in a collapse in one or the other species. The surviving species may carry some genes from the other. An example of such a case is a hybrid zone in the eastern United States, where the Blue-winged Warbler (*Vermivora cyanoptera* [previously *pinus*]) is replacing the Golden-winged Warbler (*Vermivora chrysoptera*) (Gill, 2004).

The majority of avian hybrid zones are most likely tension zones (Table 4.1). However, some avian hybrid zones seem to fit the “bounded hybrid superiority zone model”, in which hybrids are more fit compared to the parental taxa in restricted areas (Moore, 1977). The hybrid zone between Common Bulbul (*Pycnonotus barbatus*) and African Red-eyed Bulbul (*Pycnonotus nigricans*) in South Africa, for example, has been considered a bounded hybrid superiority zone (Lloyd et al., 1997). Moreover, it is possible that a hybrid zone alternates between tension zone and hybrid superiority zone dynamics. For instance, the survival and breeding success of hybrids between Medium Ground Finch (*Geospiza fortis*) and Cactus Finch (*Geospiza scandens*) on the island of Daphne Major on the Galapagos Islands differed between years, determined by ecological feeding conditions (Grant and Grant, 2006, Grant and Grant, 2008). This situation of these Darwin’s Finches is mostly not considered a hybrid zone, but it is conceivable that similar processes can occur in hybrid zones. Whether such evolutionary dynamics are more common than currently appreciated remains to be investigated, but the study of hybrid zones over multiple generations or across several transects holds promise to tackle this issue.

Table 4.1 An overview of avian hybrids zones in Europe, North America, South America, Africa, Asia and Antarctica. If possible, for each hybrid zone the location, origin (secondary or primary) and type (tension zone, moving hybrid zone or hybrid superiority zone) are given.

Species		Location (Suture Zone)	Primary (P) or Secondary (S)	Hybrid Zone Type	References
EUROPE (18)					
Phylloscopus trochilus trochilus	*P. t. acredula*	Scandinavia	S	Tension Zone	(Bensch et al., 1999)
Aquila pomarina	*A. clanga*	Central Europe	S		(Vali et al., 2010)
Corvus corone	*C. cornix*	Central Europe	S	Tension Zone	(Meise, 1928)
Ficedula albicollis	*F. hypoleuca*	Central Europe	S	Tension Zone	(Qvarnström et al., 2010)
Hippolais polyglotta	*H. icterina*	Central Europe	S	Moving Hybrid Zone	(Secondi et al., 2006)
Larus argentatus	*L. cachinnans*	Central Europe	S	Tension Zone	(Gay et al., 2007)
Luscinia megarhynchos	*L. luscinia*	Central Europe	S	Tension Zone	(Storchova et al., 2010)
Sylvia atricapilla		Central Europe	S	Tension Zone	(Mettler and Spellman, 2009)
Alectoris rufa	*A. graeca*	Alps	S	Tension Zone	(Randi and Bernard-Laurent, 1999)
Alectoris rufa	*A. chukar*	Alps	S*		(Barilani et al., 2007)
Alectoris graeca	*A. chukar*	Alps	S*		(Barilani et al., 2007)
Passer domesticus	*P. italiae*	Alps	S		(Lockley, 1992, Lockley, 1996)
Phylloscopus collybita	*P. brehmii*	Pyrenees	S	Tension Zone	(Bensch et al., 2002)
Sturnus vulgaris	*S. unicolor*	Pyrenees	S		(delaCruzCardiel et al., 1997)
Tetrao urogallus		Pyrenees	S		(Rodriguez-Munoz et al., 2007)
Larus argentatus	*L. hyperboreus*	Iceland	S		(Sternkopf et al., 2010)
Larus glaucoides complex		Iceland	S		(Weir et al., 2000)
Puffinus mauretanicus	*P. yelkouan*	Spanish Islands	S		(Genovart et al., 2012)

NORTH AND CENTRAL AMERICA (36)					
Empidonax difficilis	*E. occidentalis*	Rocky Mountains	S		(Rush et al., 2009)
Oporornis tolmiei	*O. philadelphia*	Rocky Mountains	S		(Irwin et al., 2009a)
Setophaga [Dendroica] coronata auduboni	*S. c. coronata*	Rocky Mountains	S	Tension Zone	(Brelsford and Irwin, 2009)
Setophaga virens	*S. townsendi*	Rocky Mountains	S	Tension Zone	(Toews et al., 2011)
Sphyrapicus ruber	*S. varius*	Rocky Mountains	S	Tension Zone	(Seneviratne et al., 2012)
Aphelocoma californica		Cascade-Sierra Nevada	S		(Gowen et al., 2014)
Baeolophus inornatus	*B. ridgwayi*	Cascade-Sierra Nevada	S		(Cicero, 2004)
Callipepla californica	*C. gambelli*	Cascade-Sierra Nevada	S	Tension Zone	(Gee, 2004)
Poecile gambeli		Cascade-Sierra Nevada	S		(Manthey et al., 2012)
Sphyrapicus ruber	*S. nuchalis*	Cascade-Sierra Nevada	S	Tension Zone	(Johnson and Johnson, 1985)
Strix occidentalis caurina	*S. o. occidentalis*	Cascade-Sierra Nevada	S	Tension Zone	(Barrowclough et al., 2011)
Baeolophus bicolor	*B. atricristatus*	Great Plains	S		(Curry and Patten, 2014)
Colaptes auratus auratus	*C. a. cafer*	Great Plains	S		(Moore and Buchanan, 1985)
Icterus galbula	*I. bullockii*	Great Plains	S	Tension Zone	(Carling et al., 2011)
Lanius ludovicianus migrans	*L. l. excubitorides*	Great Plains	S		(Vallianatos et al., 2001)
Passerina cyanea	*P. amoena*	Great Plains	S	Moving Hybrid Zone	(Carling and Zuckerberg, 2011)
Pipilo erythropthalmus	*P. maculatus*	Great Plains	S		(Sibley and West, 1959)
Pheucticus ludovicianus	*P. melanocephalos*	Great Plains	S	Tension Zone	(Mettler and Spellman, 2009)
Sialia sialis	*S. currucoides*	Great Plains	S		(Rounds and Munro, 1982)
Sturnella neglecta	*S. magna*	Great Plains	S		(Rohwer, 1972)
Catharus ustulatus ustulatus	*C. u. swainsoni*	Pacific Slope	S	Tension Zone	(Ruegg and Smith, 2002)
Larus occidentalis	*L. glaucescens*	Pacific Slope	S	Moving Hybrid Zone	(Gay et al., 2008)
Setophaga occidentalis	*S. townsendi*	Pacific Slope	S	Moving Hybrid Zone	(Krosby and Rohwer, 2009)
Ammodramus caudacutus	*A. nelsoni*	Great Lakes Region	S		(Walsh et al., 2011)
Poecile atricapillus	*P. carolensis*	Great Lakes Region	S	Tension Zone	(Taylor et al., 2014)

Vermivora chrysoptera	*V. pinus*	Great Lakes Region	S		(Vallender et al., 2009)
Quiscalus quiscala quiscala	*Q. q. versicolor*	Louisiana	S	Tension Zone	(Yang and Selander, 1968)
Branta canadensis	*B. hutchinsii*	Hudson Bay	S	Tension Zone	(Leafloor et al., 2013)
Rallus longirostris	*R. elegans*	Eastern USA	S		(Maley and Brumfield, 2013)
Campylorhynchus rufinucha humilis	*C. r. nigricaudatus*	Mexico	S		(Vazquez-Miranda et al., 2009)
Pipilo ocai	*P. maculatus*	Mexico	S		(Kingston et al., 2012)
Manacus candei	*M. vitellinus*	Panama	S		(Brumfield et al., 2001)
Uria lomvia	*U. aalge*	Atlantic Islands (Canada)	S		(Taylor et al., 2012)
Anas platyrhynchos	*A. wylvilliana*	Hawaii	S*		(Browne et al., 1993)
Phoebastria nigripes	*P. immutabilis*	Hawaii	S		(Rohwer et al., 2014)
Pterodroma arminjoniana	*P. neglecta*	Round Island	S		(Brown et al., 2011)
SOUTH AMERICA (15)					
Anairetes reguloides	*A. nigrocristatus*	Andes	S		(Dubay and Witt, 2014)
Cyanoliseus patagonus		Andes	S		(Masello et al., 2011)
Zimmerius viridiflavus	*Z. chrysops*	Andes	S		(Rheindt et al., 2014)
Icterus cayanensis	*I. chrysocephalus*	Amazonia	S		(D'Horta et al., 2008)
Icterus tibialis	*I. pyrrhopterus*	Amazonia	S		(D'Horta et al., 2008)
Phaeothlypis fulvicauda	*P. rivularis*	Amazonia	S		(Lovette, 2004)
Suiriri suiriri	*S. affinis*	Amazonia	S		(Hayes, 2001)
Thamnophilus caerulescens aspersiventer	*T. c. dinellii*	Amazonia	P		(Brumfield, 2005)
Thamnophilus caerulescens paraguayensis	*T. c. dinellii*	Amazonia	P		(Brumfield, 2005)
Ramphocelus flammigerus		Colombia	S*		(Bedoya and Murillo, 2012)
Camarhynchus parvulus	*C. pauper*	Galapagos	-		(Kleindorfer et al., 2014)
Geospiza fortis	*G. fuliginosa*	Galapagos	S		(Grant and Grant, 1992)
Geospiza fortis	*G. scandens*	Galapagos	S		(Grant and Grant, 1992)

Sula nebouxii	*S. variegata*	Peruvian Island	S		(Taylor et al., 2010)
Anas flavirostris	*A. georgica*	Falkland Islands	S		(McCracken and Wilson, 2011)
AUSTRALIA (20)					
Artamus cinereus albiventris	*A. c. melanops*	North	S		(Ford, 1978)
Chalcites minulitus	*C. russatus*	North	S		(Joseph et al., 2011)
Colluricincla megarhyncha parvula	*C. m. rufogaster*	North	S		(Ford, 1979)
Malurus lamberti rogersi	*M. l. assimilis*	North	S		(Ford and Johnstone, 1991)
Malurus melanocephalus cruentatus	*M. m. melanocephalus*	North	S	Tension Zone	(Lee and Edwards, 2008)
Sphecotheres viridis flaviventris	*S. v. vieilloti*	North	S		(Ford, 1982)
Artamus superciliosus	*A. personatus*	Southeast	S		(Joseph et al., 2006)
Barnardius zonarius zonarius	*B. z. barnardi*	Southeast	S		(Baker, 2010)
Daphoenositta chrysoptera leucocephala	*D. c. chrysoptera*	Southeast	S		(Short et al., 1983)
Eopsaltria australis chrysorrhoa	*E. a. australis*	Southeast	P		(Pavlova et al., 2013)
Lichenostomus melanops complex		Southeast	P		(Pavlova et al., 2014)
Malurus splendens musgravi	*M. s. melanotis*	Southeast	S		(Kearns et al., 2009)
Manorina flavigula	*M. melanotis*	Southeast	S*		(Clarke et al., 2001)
Platycercus elegans elegans	*P. e. flaveolus*	Southeast	-	"Ring Species"	(Joseph et al., 2008)
Cracticus tibicen		Southeast and West	P		(Hughes et al., 2011)
Barnardius zonarius zonarius	*B. z. semitorquatus*	West	P		(Baker, 2010)
Malurus splendens splendens	*M. s. musgravi*	West	S		(Kearns et al., 2009)
Thalassarche melanophris	*T. impavida*	New Zealand	S		(Moore et al., 2001)
Himantopus novaezelandiae	*H. himantopus*	New Zealand	S*		(Steeves et al., 2010)
Melidectes leucostephes	*M. belfordi*	New Guinea	S		(Mayr and Gilliard, 1952)

AFRICA (9)				
Campethera cailliautii cailliautii	*C. c. permista*	Western Africa	S	
Cossypha dichroa	*C. natalensis*	South Africa	S	
Pycnonotus nigricans	*P. barbatus*	South Africa	S	Hybrid Superic Zone
Phyllastrephus debilis rabai	*P. d. albigula*	Tanzania	S	
Streptopelia vinacea	*S. capicola*	Uganda	S	
Tersiphone rufiventer	*T. batesi*	Congo	S	
Tockus erythrorhynchus rufirostris	*T. e. damarensis*	Namibia	S	Tension Zon
Vidua camerunensis		Cameroon	-	
Neospiza wilkinsi	*N. acunhae*	Inaccessible Island	S	
ASIA (15)				
Circus spilonotus	*C. aeruginosus*	Siberia	S	
Corvus corone	*C. cornix*	Siberia	S	
Emberiza citrinella	*E. leucocephalus*	Siberia	S	
Lanius collurio	*L. cristatus*	Siberia	S	
Motacilla alba	*M. personatus*	Siberia	-	
Parus major complex		Siberia	-	“Ring Specie
Phylloscopus trochiloides complex		Tibet	-	“Ring Specie
Oenanthe xanthoprumna	*O. chrysopygia*	Iran	-	
Oenanthe hispanica	*O. pleschanka*	Iran	-	
Parus caeruleus	*P. cyanus*	Urals	S	
Acrocephalus arundinaceus	*A. stentoreus*	Kazakhstan	S	
Alectoris chukar	*A. magna*	Liupan Mountains	S	
Fringilla coelebs caucasica	*F. c. solomkoi*	Caucasus	S	

Pycnonotus leucotis	*P. cafer*	Pakistan	-	(Grimmett et al., 2008)
Copsychus saularis complex		Borneo - Java	S	(Sheldon et al., 2009)
ANTARCTICA (1)				
Catharacta maccormicki	*C. antarctica*		S	(Ritz et al., 2006)

* Secondary contact as a result of human disturbance

4.2.2 Formation and Distribution of Hybrid Zones

The characteristic clinal variation of hybrid zones, described in the previous section, already caught the attention of early naturalists (Chapman, 1892, Allen, 1907). They distinguished between zones of primary intergradation, where clines are the result of direct response to the environment within a single, continuous population and zones of secondary intergradation, which are the outcome of divergence in allopatry followed by secondary contact. These contrasting scenarios are now known as primary and secondary hybrid zones. Ernst Mayr (1942, 1963) argued that most hybrid zones arose through secondary contact, tightly linked with the climatic history of the Pleistocene when the ranges of many organisms were subdivided into several isolated refugia. This assumption of secondary contact was challenged by John Endler (1977), who argued that it is impossible to distinguish between differentiation along an environmental gradient (i.e. primary hybrid zone) and secondary contact, although there is strong support for the latter scenario (Hewitt, 2011).

The debate on the formation of hybrid zones is tightly connected to two modes of speciation, namely allopatric and parapatric speciation (Coyne and Orr, 2004). In allopatric speciation, the geographic range of a species is split in two or more isolated populations that diverge by natural selection or genetic drift. When allopatry is interrupted and the diverging populations have not reached complete reproductive isolation, a secondary hybrid zone can arise. Parapatric speciation concerns the evolution of reproductive isolation between populations that still exchange genes to a limited extent. This mode of speciation comes in two forms: speciation by distance and clinal speciation. In the former, gene flow is reduced as a result of isolation by distance (Mayr, 1942). Over time, the most distant populations differentiate despite a chain of interconnected populations that continue to exchange genes. A special case of speciation by distance concerns ring species, in which the chain of populations is found around a geographical barrier and the populations at the end meet without interbreeding (Irwin et al., 2001). In clinal speciation, a single population can separate into two in response to gradual spatial variation in ecological conditions (Endler, 1977). Both speciation by distance and clinal speciation can lead to the formation of a primary hybrid zone.

The development of genetic tools has shifted the emphasis of the primary-secondary hybrid zone debate from geography to gene flow (Fitzpatrick et al., 2009), thereby contrasting allopatric speciation with a model of divergence-with-gene-flow (i.e. parapatric speciation). Isolation-with-migration (IM) models provide statistical methods to infer gene flow parameters, along with population divergence times and effective population sizes (Pinho and Hey, 2010, Hey, 2010, Hey and Nielsen, 2004). Analyses of many species pairs revealed mostly divergence with essentially no gene flow (Pinho and Hey, 2010) and recent studies indicated that false positives may be common (Cruickshank and Hahn, 2014, Hey et al., 2015), suggesting that divergence-with-gene-flow (and parapatric speciation) is rare. Furthermore, even if IM models indicate a history with some gene flow, it cannot be concluded that the hybrid zone under investigation arose *in situ*. So, although IM models are useful to quantify the amount of gene flow between two diverging populations, these models alone cannot be applied to distinguish between primary and secondary hybrid zone formation.

Similar to IM models, Approximate Bayesian Computation (ABC) modelling (Beaumont, 2010)$/ has been used to estimate past effective population sizes and bottlenecks (Husemann et al., 2015, Spurgin et al., 2014b, Athrey et al., 2012, Allentoft et al., 2014), divergence times (Dolman and Joseph, 2012, Barber and Klicka, 2010) and the amount of gene flow (Illera et al., 2014). In contrast to IM models, however, ABC modelling allows for the comparison of multiple scenarios that differ in the amount and timing of gene flow. This way, it is possible to discriminate between divergence-with-gene-flow and secondary contact (Smyth et al., 2015, Raposo do Amaral et al., 2013, Yeung et al., 2011). For example, Nadachowska-Brzyska and colleagues (2013) compared 15 models (with different patterns and levels of gene flow) to assess the demographic history of Pied and Collared Flycatchers. ABC modelling based on whole genome re-sequencing data from 20 individuals supported a recent divergence with unidirectional gene flow from Pied into Collared Flycatcher, after the Last Glacial Maximum. This analysis thus indicates that the hybrid zone between these species is secondary.

Another fruitful approach in discriminating between primary and secondary hybrid zones relies on patterns of genomic divergence and linkage disequilibrium (Feder et al., 2013). During divergence-with-gene-flow there is an antagonism between selection and recombination: divergent selection builds up combinations of locally adapted genes, while recombination and gene flow break these combinations down (Gavrilets, 2004, Felsenstein, 1981). In theory, genomic features that reduce recombination, such as chromosomal inversions, may increase the effectiveness of divergent selection (Noor et al., 2001, Rieseberg, 2001). In allopatric divergence, however, there is no such antagonism between selection and recombination (Kirkpatrick and

Ravigne, 2002). As a result, allopatric populations are expected to diverge in many genomic regions via selection and drift. Based on this rationale, the following prediction can be formulated: populations undergoing divergence-with-gene-flow should be more affected by recombination, resulting in a smaller number of highly diverged regions (so-called islands of divergence) compared to allopatrically diverging populations (Via, 2001). This prediction is supported by experiments with *Timema* stick insects through the comparison of genome architecture of parapatric and allopatric populations (Nosil et al., 2012). In birds, this prediction has not been tested yet.

4.2.2.1 Secondary Contact: An Ice Age Legacy?

Allopatric speciation is regarded as the most important mode of avian speciation and consequently most avian hybrid zones are considered the outcome of secondary contact (Price, 2008). Indeed, 99 of the 114 hybrid zones in Table 4.1 are postulated to be secondary contact zones. A specific case of the allopatric speciation model, the "Pleistocene forest refugia hypothesis", states that during the colder and drier Pleistocene continuous forests were fragmented. Forest-dwelling species were isolated in these refugia and differentiated from each other. When the climate ameliorated, formerly isolated species were able to expand from the refugia (following the expansion of suitable forest habitat) and form secondary contact zones. This scenario has been proposed for Africa (Prance, 1982), South America (Haffer, 1969) and Australia (Keast, 1961), but for birds it is best documented for the Northern Hemisphere, which was largely covered in ice sheets during the Pleistocene (Pielou, 2008). Pollen records (Bennett, 1997) and fossil remains (Sommer and Zachos, 2009) indicate that several populations retreated to southern refugia, such as the Balkans, Italy or the Iberian Peninsula in Europe (Hewitt, 2011) and Florida or west Louisiana in North America (Swenson and Howard, 2005). As the climate warmed and the ice sheets retreated, populations were able to expand their ranges northwards (a process known as leading edge expansion) and establish secondary contact zones in the process (Hewitt, 2011).

The majority of avian hybrid zones in North America and Europe appear to be the result of secondary contact after expansion from Pleistocene refugia. However, multiple lines of evidence are necessary to confidently adhere to a scenario of post-Pleistocene range expansion and secondary contact. Ideally, fossils confirm that certain populations resided in the postulated refugia during the Pleistocene (Sommer and Zachos, 2009), but such direct evidence mostly lacks for birds, because of the rarity of avian fossilization (Lyman, 1994). Indirect evidence involves (pre-)Pleistocene divergence times (Weir and Schluter, 2004, Klicka and Zink, 1997) and genetic signatures of post-Pleistocene range expansion (Ruegg and Smith, 2002). In addition, ecological niche modelling can be applied to reconstruct possible expansion routes, although this method can be fraught with difficulties because changed ecological circumstances cannot easily be incorporated into the model (Ruegg et al., 2006, Cicero, 2004, Manthey et al., 2012). In some cases, divergent migratory strategies of the interbreeding species (i.e. migratory divide) can provide extra support for a Pleistocene refugial scenario if the migration routes coincide with the hypothesized post-Pleistocene expansion routes (Ruegg and Smith, 2002, Bensch et al., 2009). Only a handful of studies have provided these multiple lines of evidence (Cicero, 2004, Manthey et al., 2012, Ruegg et al., 2006).

4.2.2.2 Primary Contact: Ring Species and Clinal Variation

Parapatric speciation (and the *in situ* formation of primary hybrid zones) seems less common in birds compared to allopatric speciation (Price, 2008): only six of the 114 hybrid zones in Table 4.1 were considered primary contact zones. As discussed above, parapatric speciation can give rise to primary hybrid zones in two ways: isolation by distance and clinal speciation. Examples of primary hybrid zone formation as a result of isolation by distance mainly concern ring species, which can be characterized by four criteria (Irwin et al., 2001): (1) coexistence of the two distinct forms at the ends of the ring, (2) gene flow through the chain of connected populations, (3) true geographic ring without gaps, and (4) terminal populations connected by gradual geographic variation. Not all putative avian ring species conform to these criteria (Päckert et al., 2005, Liebers et al., 2004, Alcaide et al., 2014). Hence, not every hybrid zone within a putative ring species complex can automatically be regarded as a primary hybrid zone, as there may have been historical breaks in gene flow, in which case some hybrid zones are actually secondary (Alcaide et al., 2014, Kvist et al., 2003, Päckert et al., 2005). In addition, several more detailed scenarios for the formation of a ring species have been proposed (Smyth et al., 2015). A promising approach to discriminate between these various scenarios of ring formation and to determine whether the hybrid zones along the ring are of primary origin is ABC modelling (discussed above). For example, using ABC modelling in combination with ecological niche modelling, Smyth and colleagues (2015) showed that the most likely scenario for the ring formation around the Sierra Nevada in Song Sparrow (*Melospiza melodia*) is via a process of isolation and subsequent ecological divergence after secondary contact.

A few possible cases of primary hybrid zone formation by clinal speciation in birds have been documented (Cheviron and Brumfield, 2009, Ribeiro et al., 2011, Brumfield, 2005). Especially, the south-eastern part of Australia, which is characterized by strong climatic gradients, houses some examples of possible clinal speciation, involving *Platycercus* Parrots (Joseph et al., 2008), *Cracticus* Magpies (Toon et al., 2003) and *Lichenostomus* Honeyeaters (Pavlova et al., 2014). A well-documented case concerns the Eastern Yellow Robin (*Eopsaltria australis*), of which parapatric populations exhibit divergent mtDNA whereas nuclear differentiation is low; this pattern can be explained by selection on mtDNA along an environmental gradient in the face of nuclear gene flow (Morales et al., 2015, Pavlova et al., 2013). An integrated study of avian hybrid zones in this area of Australia could provide important insights into the dynamics of parapatric speciation and the formation of primary hybrid zones.

4.2.2.4 Distribution of Hybrid Zones: Suture Zones

Hybrid zones seem to cluster in certain geographic regions (Swenson and Howard, 2004, 2005). With inclusion of phylogeographic breaks (i.e. geographically and genealogically separated clades that come into contact in narrow regions, Irwin, 2002) and contact zones without hybridization, these regions are known as suture zones. In North America, Remington (1968) proposed six major and seven minor suture zones, of which only a few coincide with actual hotspots of avian hybrid zones (Swenson and Howard, 2004, 2005). Suture zones have also been suggested for Europe (Hewitt, 2011), South America (Naka et al., 2012) and Australia (Moritz et al., 2009).

The mechanisms of hybrid zone formation mentioned above (expansion from Pleistocene refugia, primary hybrid zone formation and anthropogenic disturbance) have also been invoked to explain the clustering of hybrid zones, by assuming that hybrid zones that are part of the same suture zone are the result of a common underlying mechanism. But close inspection of several hybrid zones within a putative suture zone reveals that each hybrid zone is species-specific and not generic. Although the large-scale patterns are often the result of expansion from shared Pleistocene refugia, fine-scale characteristics of the hybrid zones, such as the exact location and stability, are determined by dissimilar ecological and climatic gradients within the respective suture zones. For example, in the Cascade-Sierra Nevada suture zone in western North America, the hybrid zone between *Baeolophus* titmice is located on an ecological transition from oak forest in the west to pinyon-juniper woodlands in the east (Cicero, 2004), whereas California Quail (*Callipepla californica*) and Gambel's Quail (*Callipepla gambelii*) interbreed along a climatic gradient of decreasing rainfall and increasing temperature, independent of forest type (Gee, 2004). This example shows that one has to be cautious when making statements about one hybrid zone based on the characteristics of other hybrid zones in the same suture zone. As mentioned before, it is important to keep in mind that most if not all hybrid zones are species-specifically determined.

4.3. Patterns of Introgression

Introgression is the incorporation of genetic material from one (sub)species into the gene pool of another by means of hybridization and backcrossing (Arnold, 2006). The slow evolution of intrinsic postzygotic isolation in birds enables backcrossing and thus increases the potential for introgression (Fitzpatrick, 2004). Indeed, numerous studies have documented the exchange of genetic material between bird species (Tables 4.2 and 4.3) and it has been argued that introgression is a critical (and often neglected) factor in avian speciation (Rheindt and Edwards, 2011). The increasing sensitivity of detection of introgression with genetic methods (Section 4.3.1) has uncovered several striking introgression patterns, such as differential introgression among autosomal loci, mtDNA and sex-linked loci (Section 4.3.2) and asymmetrical gene flow from one species into the other (Section 4.3.3). In the following sections, we explore these patterns and review the proposed mechanisms.

4.3.1 Detecting Introgression

The toolkit for detecting introgression has expanded over the years and the development of new methods in avian biology closely follows the progress in molecular markers, from allozymes, through microsatellites and mtDNA, to genomics (Kraus and Wink, 2015).

Early studies on introgression focused on traditional population genetic parameters to quantify sequence divergence, such as F_{ST} (Nei, 1977) or Nei's *D* (Nei, 1978). These parameters could easily be calculated from allozymic data (e.g., Bell, 1996, Saino et al., 1992). The development of molecular markers by PCR led to an increased sensitivity in introgression analysis. A pivotal study was conducted by Tegelström and Gelter (1990) on *Ficedula* flycatchers, in which they compared divergence levels in mitochondrial and nuclear markers. They stated that "the low nuclear differentiation is explained by sex-biased gene flow and introgression in nuclear genes (via fertile male hybrids), while the high mitochondrial sequence divergence is preserved by the sterility of hybrid females, which prevents mitochondrial introgression."

The study of hybrid zones in combination with the application of geographical cline theory boosted the interest in hybridization research (Barton and Hewitt, 1985), and led to the publication of some classical papers on *Bombina* fire-bellied toads (Szymura and Barton, 1986) and *Chorthippus* grasshoppers (Butlin and Hewitt, 1985). Cline theory provides a framework to analyse changes in traits or allele frequencies as a function of geographic distance across a hybrid zone transect. Several characteristics of the observed clines can be used to make inferences about hybrid zone dynamics. For instance, cline width in combination with dispersal rates allows estimation of selection pressures (Barton and Gale, 1993). Alleles and traits under similar selective pressures will show concordant cline widths and centres, whereas those subject to different selection pressures will show displaced cline centres compared to the majority of the other clines (Barton, 1983).

The implementation of Bayesian Admixture Analysis in the software package STRUCTURE (Pritchard et al., 2000) provided a powerful tool for detecting introgression and instigated a marked increase in avian introgression studies. This method uses multilocus genotype data and is based on a clustering algorithm that assigns individuals to populations. Hence, this method can detect introgression on an individual level, in contrast to cline analyses which detect introgression on the locus level. Most studies used microsatellites, often in combination with mitochondrial markers (e.g., Barilani et al., 2005). The rapid progress in sequencing techniques introduced the application of SNPs and other genome-wide markers to Bayesian Admixture Analysis (Saetre et al., 2003, Kraus et al., 2012). STRUCTURE, however popular it remains since over a decade, has limitations with respect to the underlying population genetic model, such as adherence to Hardy Weinberg and linkage equilibria (Jombart et al., 2010). Alternatives to STRUCTURE have indeed been developed to address these issues, such as ADMIXTURE (Alexander et al., 2009) and Discriminant Analysis of Principle Components or DAPC (Jombart et al., 2010). Eventually, STRUCTURE, ADMIXTURE and DAPC (and other similar software) are best utilized alongside each other (Frosch et al., 2014).

A more recent development is the implementation of Isolation-with-Migration (IM) models, already mentioned above (Hey and Nielsen, 2004, Hey and Nielsen, 2007). These models rely on mathematical coalescent theory (Kingman, 1980, Wakeley, 2009) and provide a way to calculate migration rates (as a proxy for introgression) between populations using multilocus genotype data (Peters et al., 2007, Irwin et al., 2009a). An offshoot of coalescent theory is the D-statistic (Durand et al., 2011), a statistical test that was first employed to quantify the amount of genetic exchange between Neanderthals and modern humans (Green et al., 2010). The D-statistic exploits the asymmetry in frequencies in two nonconcordant gene trees in a three-population setting. Eaton and Ree (2013) have expanded this technique to four taxa, which creates the possibility to determine the direction of introgression. To date, only two studies on avian introgression have yet adopted this method to study introgression patterns in *Zimmerius* flycatchers (Rheindt et al., 2014) and Darwin's Finches (Lamichhaney et al., 2015).

Apart from these methods, discordance between phylogenetic gene trees can be used to detect introgression (Andersson, 1999, Weckstein et al., 2001). However, such discordance can also be the result of incomplete lineage sorting (Maddison, 1997). Disentangling introgression from incomplete lineage sorting can be challenging (Funk and Omland, 2003), but several approaches have been proposed (reviewed in Toews and Brelsford, 2012).

4.3.2 Comparison of Genomic Classes

Loci can be divided into three different major genomic classes based on their mode of inheritance, namely autosomal, sex-linked and mitochondrial loci. The different modes of inheritance are expected to lead to differences in introgression potential. Two processes, Haldane's Rule and sex-biased dispersal, have been proposed to explain differential patterns of introgression between genomic classes. In the following paragraphs, we explore the generality of these proposed mechanisms.

The mechanisms underlying Haldane's Rule (1922) have been invoked to explain differential introgression among autosomal, sex-linked and mitochondrial loci. Haldane's Rule states that "when in the F_1 offspring of two different animal races one sex is absent, rare, or sterile, that sex is the heterozygous [or heterogametic] sex". In birds, where sex is determined by a ZZ/ZW system, females are the heterogametic sex and hybrid females are thus expected to show greater fitness reductions compared to male hybrids. This expectation has been confirmed for birds in general (Price and Bouvier, 2002), but also for specific bird groups, including ducks (Tubaro and Lijtmaer, 2002), galliform birds (Arrieta et al., 2013), and pigeons and doves (Lijtmaer et al., 2003). Consequently, introgression of maternally inherited loci, such as mitochondrial and W-linked loci, will be impaired by unfit (e.g., sterile) females. Indeed, lower introgression rates for mtDNA compared to autosomal loci have been reported for 21 hybridizing bird species pairs (Table 4.2).

Haldane's Rule also predicts lower introgression rates for Z-linked loci compared to autosomal loci. This prediction relies on dominance theory, one of the possible mechanisms that have been invoked to explain Haldane's Rule. Dominance theory is based on the Dobzhansky-Muller incompatibility model (Wu and Ting, 2004, Presgraves, 2010) and states that hybrid sterility and inviability arise from the interaction of two genes that have developed incompatible alleles during an allopatric phase. If these alleles are recessive and located on the Z-chromosome, their effect will be much larger in female birds because they lack another Z-chromosome that could carry a dominant version of the incompatibility allele, which would nullify the negative effect of the recessive allele.

It has been suggested that the Z-chromosome (and its equivalent in mammals, the X-chromosome) plays a disproportionately large role in the development of intrinsic postzygotic isolation (Presgraves, 2008). Several lines of evidence support this "Large Z-effect." First, Z-linked genes evolve faster compared to autosomal loci ("Faster Z-effect") which might speed up the accumulation of incompatibility alleles on this sex chromosome (Ellegren, 2009, Storchova et al., 2010). Second, if genes involved in premating and postzygotic isolation both arise on the Z-chromosome and thus become physically linked, it is expected to facilitate the evolution of isolation barriers by means of reinforcement (Hall and Kirkpatrick, 2006). The latter situation has been described for *Ficedula* flycatchers, where genes for low hybrid fitness and female preference are located on the Z-chromosome (Saetre et al., 2003, Backström et al., 2010). The important role of the Z-chromosome in reproductive isolation is expected to result in lower rates of introgression compared to autosomal loci. This pattern has been documented for three bird species pairs so far (*Aquila*, Backström and Vali, 2011, *Passerina*, Carling and Brumfield, 2008, *Luscinia*, Storchova et al., 2010).

All in all, most avian introgression studies are in line with the predictions of Haldane's rule, but to confidently attribute the observed introgression pattern to Haldane's rule, one has to provide convincing evidence for reduced fitness of hybrid females (compared to hybrid males), which only a few studies were able to do. For instance, hatching success of hybrids between Collared Flycatcher and Pied Flycatcher confirmed that hybrid males are fertile in contrast to hybrid females (Gelter et al., 1992). Similarly, crosses in captivity between Thrush Nightingale (*Luscinia luscinia*) and Common Nightingale (*Luscinia megarhynchos*) yielded sterile females and fertile males (Stadie, 1991). Finally, an extensive mark-and-recapture study in the Polish hybrid zone between Herring Gull (*Larus argentatus*) and Caspian Gull (*Larus cachinnans*) showed that hybrid females have lower survival rates compared to hybrid males and pure individuals (Neubauer et al., 2014).

Another process that can account for differential introgression of genomic classes is sex-biased dispersal. The rationale is as follows: "If a species expands its range and meets a closely related species with which reproductive barriers are still incomplete, asymmetric introgression will take place from the local into the colonizing species. […] However, if there is sufficient intraspecific gene flow among populations of the colonizing species, genetic drift will be reduced and introgressed alleles will be less likely to increase in frequency by chance in the colonizing species" (Petit and Excoffier, 2009). Hence, there should be a negative correlation between rate of introgression and rate of intraspecific gene flow for a given genomic class. In other words, if the female is the dispersing sex (which results in high rates of intraspecific mitochondrial gene flow), this will lead to lower levels of mtDNA introgression compared to autosomal loci, and vice versa, if the male is the dispersing sex. Nine studies conform to the expected pattern (Table 4.2), but whether this explanation is correct remains to be tested, for instance by quantifying levels of intra- and interspecific gene flow in a meta-analysis. This knowledge gap provides promising avenues for further research.

In summary, most introgression patterns are in line with the predictions of Haldane's rule, the sex-biased dispersal hypothesis or both. These mechanisms are not mutually exclusive and could possibly enhance each other. But to confidently attribute the observed patterns to either of these processes, one has to provide additional evidence, such as reduced fitness of females or intraspecific gene flow rates. Most studies do not to deliver these crucial pieces of evidence and remain trapped in speculation.

Table 4.2 Patterns of Introgression for different genomic classes: mitochondrial (mtDNA), autosomal (nDNA) and sex-linked (Z) loci. The observed patterns can be in line with the predictions of Haldane's Rule and/or sex-biased dispersal.

Species		Introgression Pattern	In line with prediction of		References
			Haldane's Rule	Sex-biased Dispersal*	
Aquila clanga	*Aquila pomarina*	nDNA > mtDNA	YES		(Helbig et al., 2005)
		nDNA > Z	YES		(Backström and Vali, 2011)
Aquila adalberti	*Aquila heliaca*	nDNA > mtDNA	YES		(Martinez-Cruz and Godoy, 2007)
Anas zonorhyncha	*Anas platyrhynchos*	mtDNA > nDNA	NO	YES	(Kulikova et al., 2004)
Anas crecca	*Anas carolensis*	nDNA > mtDNA	YES	NO	(Peters et al., 2012)
Branta canadensis	*Branta hutchinsii*	nDNA > mtDNA	YES	NO	(Leafloor et al., 2013)
Large white-headed Gulls (Larus)		nDNA > mtDNA	YES	YES	(Crochet et al., 2003)
Larus argentatus	*Larus cachinnans*	nDNA > mtDNA	YES	YES	(Gay et al., 2007)
Larus argentatus	*Larus hyperboreus*	nDNA > mtDNA	YES	YES	(Vigfusdottir et al., 2008)
Larus smithsonianus	*Larus marinus*	mtDNA > nDNA	NO	NO	(Pons et al., 2014)
Lagopus lagopus	*Lagopus muta*	nDNA > mtDNA	YES	YES	(Quintela et al., 2010)
Puffinus mauretanicus	*Puffinus yelkouan*	nDNA > mtDNA	YES	YES	(Genovart et al., 2012)
Sula variegata	*Sula nebouxii*	nDNA > mtDNA	YES		(Taylor et al., 2013)
Hippolais icterina	*Hippolais polyglotta*	nDNA > mtDNA	YES		(Secondi et al., 2006)
Passerina amoena	*Passerina cyanea*	nDNA > Z > mtDNA	YES		(Carling and Brumfield, 2008)
Aphelocoma californica		nDNA > mtDNA	YES		(Gowen et al., 2014)
Zonotrichia capensis (subspecies)		nDNA > mtDNA	YES	YES	(Cheviron and Brumfield, 2009)
Icterus galbula	*Icterus bullockii*	nDNA > mtDNA	YES		(Jacobsen and Omland, 2012)
Luscinia megarhynchos	*Luscinia luscinia*	nDNA > Z	YES		(Storchova et al., 2010)

Ficedula hypoleuca	*Ficedula albicollis*	nDNA > mtDNA	YES	YES	(Tegelström and Gelter, 1990)
Baeolophus inornatus	*Baeolophus ridgwayi*	nDNA > mtDNA	YES		(Cicero, 2004)
Poecile atricapillus	*Poecile carolensis*	nDNA > mtDNA	YES		(Davidson et al., 2013)
Vermivora pinus	*Vermivora chrysoptera*	nDNA > mtDNA	YES		(Vallender et al., 2007)
Phylloscopus collybita	*Phylloscopus brehmii*	nDNA > mtDNA	YES	YES	(Helbig et al., 2001)
Empidonax difficilis	*Empidonax occidentalis*	nDNA > mtDNA	YES		(Rush et al., 2009)

*** Dispersal patterns based on Greenwood (1980) and Clarke et al. (1997)**

4.3.3 Asymmetric Introgression

The comparison of introgression rates between genomic classes has yielded important insights into the dynamics of hybridization. However, the advent of next-generation sequencing techniques revealed considerable variation in introgression rates among individual loci, regardless of the genomic class to which they belong (e.g., Carling and Brumfield, 2008, Parchman et al., 2013, Baldassarre et al., 2014). The extent of introgression of a specific allele depends on several factors, such as hybrid fitness, reproductive isolation and genetic linkage (Wu, 2001, Payseur, 2010, Barton, 1979). Alleles can be roughly divided into three categories: (1) neutral alleles that are free to flow between species, (2) alleles that confer an adaptive advantage and introgress quickly, and (3) alleles that lead to reduced fitness and inhibit gene flow. Hybrid genomes are a mosaic of these three categories, mingled by migration and recombination (Payseur, 2010, Wang et al., 2011). Hence, most species boundaries are semipermeable: some genomic regions (e.g., those leading to reduced hybrid fitness) show restricted gene flow while other regions (e.g., comprising neutral or advantageous alleles) are allowed to flow freely (Barton and Hewitt, 1985).

Asymmetric introgression is the situation in which alleles flow primarily from one species' gene pool into the other. This introgression pattern is quite common in birds (Table 4.3) and the underlying mechanisms are manifold (reviewed in Wirtz, 1999). Asymmetric introgression patterns can be the result of chance processes, such as neutral diffusion of alleles across a cline (Barton, 1979) or random lineage extinction (Wilson et al., 1985), but mostly species-specific differences in demography and behaviour (e.g., female choice, forced copulations and brood amalgamation) are involved (Figure 4.1).

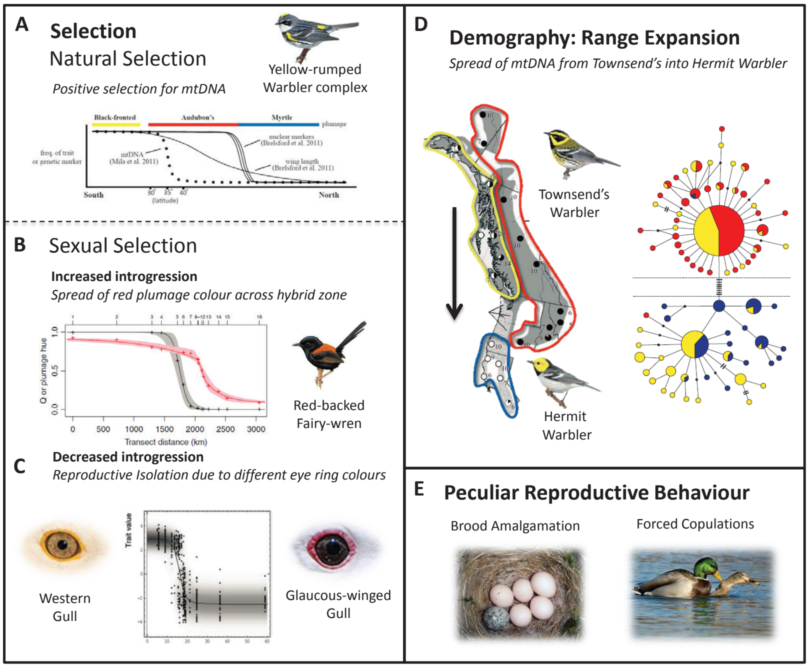


Figure 4.1 Mechanisms that can lead to asymmetric introgression. Three cline analyses illustrate the influence of natural and sexual selection on introgression. (A) In the Yellow-rumped Warbler complex positive selection on mtDNA has led to introgression from Myrtle Warbler into Audubon's Warbler (dotted line, Mila et al., 2011). (B) Hybridization between Red-backed Fairy-wren (*Malurus melanocephalus cruentatus*) and Orange-backed Fairy-wren (*M. m. melanocephalus*) in Australia has resulted to the introgression of red plumage colour into the Orange-backed subspecies (red line), because females prefer red males (Baldassarre et al., 2014). (C) The Herring Gull (*Larus argentatus*) and the Caspian Gull (*L. cachinnans*), two large gull species that interbreed in Poland, are reproductively isolated by eye ring colour, resulting in reduced introgression of these traits and thus a steep cline (Gay et al. 2007). (D) the Townsend's Warbler (*Setophaga townsendi*) is expanding rapidly across Northern America, where it is replacing the Hermit Warbler (*S. occidentalis*). This range expansion results in asymmetric introgression, as shown in the mtDNA haplotype network. (E) Certain reproductive behaviours, such as brood amalgamation and forced copulations, can also explain patterns of asymmetric introgression.

4.3.3.1 Demography

Often, demographic processes are sufficient to explain the occurrence of asymmetric introgression. When discussing the occurrence of natural hybridization in fish, Hubbs (1955) remarked that "great scarcity of one species coupled with the abundance of another often leads to hybridization: the individuals of the sparse species seem to have difficulty in finding their proper mates." This observation was referred to as the "desperation hypothesis", but is currently known as Hubbs' principle. Because the resulting hybrids are most likely to backcross with members of the most abundant species, the direction of gene flow will be from the rare into the abundant species. In the Falkland Islands, Speckled Teals (*Anas flavirostris*) outnumber Yellow-billed Pintails (*Anas georgica*) about ten to one. This numerical imbalance can explain the asymmetrical gene flow from Yellow-billed Pintail into Speckled Teal (McCracken and Wilson, 2011). Similar cases have been described for Mallard and Eastern Spot-billed Ducks in Eastern Russia (Kulikova et al., 2004) and *Streptopelia* doves in Uganda (den Hartog et al., 2010).

A difference in species abundance can be the result of expansion of one species into the range of another. Initially, the expanding species is outnumbered and is thus more likely to engage in heterospecific matings. As the expansion proceeds, the resident species and previously produced hybrids are engulfed by the expanding species, thereby overturning the numerical imbalance. Consequently, hybrids have a higher chance of backcrossing into members of the expanding species, resulting in a genetic wake of introgressed genes following the wave front of the expanding species (Krosby and Rohwer, 2009, Secondi et al., 2006, Buggs, 2007). A simulation study of this scenario showed that introgression of neutral alleles is almost exclusively from the resident into the invading species (Currat et al., 2008). This introgression pattern can be attributed solely to the demographic imbalance between two species, but the inclusion of interspecific competition amplifies the rate of introgression. For example, the Townsend's Warbler (*Setophaga townsendi*) is expanding across western Northern America, where it is replacing the Hermit Warbler (*Setophaga occidentalis*) (Figure 4.1D, Krosby and Rohwer, 2010). This rapid expansion was attributed to the competitive superiority of the Townsend's Warbler in terms of clutch size (Pearson and Rohwer, 1998), breeding success (Pearson, 2000) and aggression (Pearson and Rohwer, 2000, Owen-Ashley and Butler, 2004). Table 4.3 contains nine cases where asymmetrical introgression can be attributed to range expansion.

4.3.3.2 Natural and Sexual Selection

Alleles may be under direct positive selection or they may be linked to favourably selected ones and consequently hitchhike during a selection event (Barton, 2000). For instance, the majority of Audubon's Warblers (*Setophaga auduboni*) possess Myrtle Warbler (*S. coronata*) mtDNA (mtDNA does not undergo recombination and therefore all of its alleles are linked) (Brelsford et al., 2011). Cline analyses showed that the transition between this mtDNA haplotype and that of the Black-fronted Warbler (*S. nigrifrons*) largely coincided with a change in migratory behaviour: northern Audubon's Warbler and Myrtle Warbler migrate to Central America, while the Black-fronted Warbler is resident (Mila et al., 2011). Possibly, natural selection on one of the mtDNA alleles favoured the complete Myrtle Warbler mtDNA genome that introgressed into migratory Audubon's Warblers (Figure 4.1A). This "migration adapted mitochondrion" hypothesis is further supported by the fact that Myrtle-type mitochondria are metabolically more efficient compared to Black-fronted-type ones (Toews et al., 2014). Complete mitochondrial replacement between Yellowhammers (*Emberiza citrinella*) and Pine Buntings (*E. leucocephalos*) has also been attributed to strong natural selection (Irwin et al., 2009b).

Sexual selection can lead to differential introgression of alleles too, as exemplified by several cases of asymmetrical introgression involving secondary sexual characteristics, such as plumage (Stein and Uy, 2006, Baldassarre et al., 2014). For instance, hybridization between Red-backed Fairy-wren (*Malurus melanocephalus cruentatus*) and Orange-backed Fairy-wren (*Malurus m. melanocephalus*) across the Carpentarian Barrier in Australia has led to the introgression of red plumage colour into the Orange-backed subspecies (Figure 4.1B, Baldassarre et al., 2014). An experimental study showed that artificially reddened males have significantly more extra-pair offspring compared to orange males, which suggests that greater reproductive success of red males drives the observed introgression (Baldassarre and Webster, 2013). A similar situation has been described in Panama, where yellow plumage colour of Golden-collared Manakin (*Manacus vitellinus*) has spread into the populations of the White-collared Manakin (*Manacus candei*) by means of strong sexual selection at mixed leks, where males of both species gather to attract mates (McDonald et al., 2001, Parsons et al., 1993, Stein and Uy, 2006). Golden-collared males mated more compared to the white-collared ones, but only if the frequency of golden-collared birds is higher than white-collared birds in the lek (Stein and Uy, 2006).

White-collared Manakins and Orange-backed Fairy-wrens thus prefer yellow and red males, respectively, even if it involves a heterospecific bird. Relaxed female choice or preference for heterospecifics has been documented for other bird species as well (Wirtz, 1999). Asymmetric introgression from Thick-billed (*Uria lomvia*) into Common Murres (*U. aalge*) is most likely the result of differences in female choice. Female Thick-billed Murres might prefer to mate with male Common Murres because of their elaborate rituals during the breeding season. In addition, male Common Murres are larger than male Thick-billed Murres, which might provide them with a competitive advantage (Taylor et al., 2012). Similarly, asymmetric hybridization between Greater (*Aquila clanga*) and Lesser Spotted Eagle (*A. pomarina*) can be attributed to assortative mating by size (Helbig et al., 2005).

When secondary sexual characteristics play an important role in assortative mate choice, sexual selection can also lead to decreased levels of introgression. In seabirds, it has been suggested that the colouration of bare parts, such as bill or feet, plays an important role in mate choice (Pierotti, 1987). It is thus expected that these phenotypic characters will show low levels of introgression when hybridization occurs. This expectation has been confirmed for Herring Gull (*Larus argentatus*) and Caspian Gull (*L. cachinnans*), two large gull species that interbreed in Poland. Introgression rates of phenotypic characters, such as iris and eye-ring colour, were very low compared to neutral genetic markers (Gay et al., 2007). Birds with dark irises and eye-rings (*L. cachinnans*) are thus partly reproductively isolated from birds with light coloured irises and eye-rings (*L. argentatus*) due to premating barriers (Figure 4.1C).

4.3.3.3 Brood amalgamation and forced copulations

Peculiar reproductive strategies, such as interspecific forced copulations and brood amalgamation, have been invoked to explain asymmetric hybridization (Figure 4.1E). For instance, Black-footed Albatrosses (*Phoebastria nigripes*) were more successful in heterospecific rape attempts compared to Laysan Albatrosses (*P. immutabilis*), which probably resulted in asymmetric gene flow from Black-footed into Laysan Albatrosses (Rohwer et al., 2014). Forced copulations are also common in waterfowl (Mckinney et al., 1983), but a meta-analysis found more support for interspecific brood amalgamation than for forced copulations as a source for hybrids (Randler, 2005). Interspecific brood amalgamation entails the laying of eggs in heterospecific nests. When the eggs hatch, the fledglings are imprinted on another species and might prefer mates of this species in later life. This mechanism was tested experimentally by Fabricius (1991), who placed Greylag Goose (*Anser anser*) eggs in the nests of Canada Geese (*Branta canadensis*). The next year, several male Greylag Geese that were fostered by Canada Geese returned to the breeding grounds accompanied by a Canada Goose mate.

4.3.3.4 Asymmetric Postzygotic Isolation

Finally, asymmetry in postzygotic isolation mechanisms can lead to patterns of differential gene flow. When one type of cross is less fit compared to the reciprocal cross, introgression will be biased in the next generation. In field crickets, for example, hybrids between male *Gryllus firmus* and female *G. pennsylvanicus* are fertile, while the reciprocal cross is unable to reproduce (Harrison, 1983). For birds, such cases have not been documented yet, although captive hybrids between male Ring-necked Doves (*Streptopelia capicola*) and female Vinaceous Doves (*S. vinacea*) exhibited slightly higher fertility compared to the reciprocal cross (den Hartog et al., 2010).

4.3.4 Anthropogenic Introgression

The processes described above encompass natural causes of introgressive hybridization. However, introgression can also be the result of recent anthropogenic disturbance, a process that Edgar Anderson (1948, 1949) termed "Hybridization of the Habitat." Several cases of avian hybridization took place in the last century due to human activities, such as habitat modification and introduction of non-native species (Barilani et al., 2007, Bedoya and Murillo, 2012, Clarke et al., 2001).

The introduction of non-native species into the habitat of closely related species has led to several hybridization events. Specifically, the massive releases of captive-reared game birds (mostly galliform birds and waterfowl) have created conservation issues regarding hybridization (Randi, 2008). The introduction of Mallards from European game-farm stocks in North America and Australia has resulted in hybridization with several endemic species, such as the American Black Duck (*Anas rubripes*) (Mank et al., 2004), the Grey Duck (*Anas superciliosa superciliosa*) in New Zealand

(Rhymer et al., 1994) and the Hawaiian Duck (*Anas wylvilliana*) or Koloa (Fowler et al., 2009). In addition, huge numbers of farm-reared Mallards are interbreeding with their wild conspecifics. The low genetic diversity of the captive birds threatens the genetic integrity of the wild populations in Sweden, France and the Czech Republic (Cizkova et al., 2012, Champagnon et al., 2013). A well-known case of waterfowl hybridization involves the Ruddy Duck (*Oxyura jamaicensis*) which was introduced to the UK in the 1950s and spread to Spain where it hybridized with the endangered White-headed Duck (*Oxyura leucocephala*). Intensive management efforts, including the culling of hybrids, have successfully controlled the invasion of the Ruddy Duck in Europe (Munoz-Fuentes et al., 2013).

Red-legged Partridge (*Alectoris rufa*) and Rock Partridge (*Alectoris graeca*) are extensively hunted in southern Europe and large numbers of captive-reared partridges, often involving non-native Chukars (*Alectoris chukar*) and hybrids, have been released into the wild to counteract population declines. These restockings have led to extensive introgressive hybridization between Chukar, Red-legged Partridge and Rock Partridge (Barilani et al., 2007). A similar situation concerns *Coturnix* quails, where Japanese Quails (*Coturnix japonica*) were released to supplement populations of Common Quails (*Coturnix coturnix*) for hunting purposes. Barilani et al. (2005) reported that about 9% of the quails sampled in Italy and Spain were hybrids and a genetic study in France detected low levels of introgression (Chazara et al., 2010). The effects of released birds on the native population of Common Quails seem relatively limited given the massive restocking (e.g., over one million Japanese Quails and hybrids released in Spain between 1990 and 2006) and the presence of hybrids in the captive stock (Puigcerver et al., 2007). The low success of farmed birds and hybrids can be attributed to the low probability of hybrid females to survive long enough to breed and high levels of nest predation when they do succeed in breeding (Puigcerver et al., 2014). Limited genetic exchange following restocking has also been reported for *Perdix* Partridges (Andersen and Kahlert, 2012, Liukkonen et al., 2012), *Numida* Guineafowl (Walker et al., 2004) and Turkey (*Meleagris gallopavo*) subspecies (Latch et al., 2006).

Habitat modification and land use changes can enable the expansion of one species into the range of another and possibly lead to hybridization. For instance, the Lesser Spotted Eagle (*Aquila pomarina*) and the endangered Greater Spotted Eagle (*A. clanga*) use different areas for breeding and hunting, but some landscape changes have enabled these species to inhabit the same area and interbreed (Maciorowski and Mirski, 2014). Similarly, the New Zealand endemic Black Stilt (*Himantopus novaezelandiae*) hybridizes with the Pied Stilt (*Himpantopus himantopus leucocephalos*), which was able to colonize New Zealand due to extensive deforestation and modification of riverine areas (Steeves et al., 2010). In southeast Australia, vegetation clearance in the 1950s has led to hybridization between Black-eared Miner (*Manorina melanotis*) and Yellow-throated Miner (*Manorina flavigula*), two formerly allopatric species (Clarke et al., 2001). Rhymer and Simberloff (1996) even argue that the management of forests on the Great Plains of North America has resulted in the creation of stepping stones and corridors for several bird taxa. This would mean that anthropogenic processes are responsible for the numerous hybrid zones in this area (Rising, 1983).

Table 4.3 Patterns of asymmetric introgression from one species into the other for particular marker types (mtDNA and nDNA) or plumage traits. These patterns can be explained by several mechanisms, such as sexual selection, Hubb's principle, range expansion, hybrid zone movement or migration.

Species		Introgressed Marker Type	Proposed Mechanism	References
From	**To**			
Aquila clanga	*A. pomarina*	mtDNA	Sexual Selection	(Helbig et al., 2005)
Aquila heliaca	*A. adalberti*	nDNA	Hubb's Principle	(Martinez-Cruz and Godoy, 2007)
Anas platyrhynchos	*A. superciliosa*	mtDNA	Sexual Selection	(Rhymer et al., 1994)
Anas platyrhynchos	*A. zonorhyncha*	mtDNA – nDNA	Range Expansion	(Kulikova et al., 2004)
Anas platyrhynchos	*A. fulvigula*	mtDNA – nDNA		(Peters et al., 2014a)
Anas falcata	*A. strepera*	mtDNA		(Peters et al., 2007)
Anas wylvilliana	*A. platyrhynchos*	mtDNA	Sexual Selection	(Fowler et al., 2009)
Anas georgica	*A. flavirostris*	mtDNA – nDNA	Hubb's Principle	(McCracken and Wilson, 2011)
Anas crecca	*A. carolensis*	nDNA		(Peters et al., 2012)
Branta canadensis	*B. hutchinsii*	mtDNA		(Leafloor et al., 2013)
Uria lomvia	*U. aalge*	mtDNA	Sexual Selection	(Taylor et al., 2012)
Larus argentatus	*L. cachinnans*	mtDNA	Sexual Selection	(Gay et al., 2007)
Larus hyperboreus	*L. argentatus*	mtDNA		(Vigfusdottir et al., 2008)
Larus smithsonianus	*L. marinus*	mtDNA		(Pons et al., 2014)
Stercorarius pomarinus	*S. skua*	mtDNA	Range Expansion	(Andersson, 1999)
Streptopelia capicola	*S. vinacea*	mtDNA	Hubb's Principle	(den Hartog et al., 2010)
Platalea leucocorodia leucocorodia	*P. l. balsaci*	nDNA	Dispersal between Colonies	(den Hartog et al., 2010)
Phoebastria nigripes	*P. immutabilis*		Heterospecific Rape	(Rohwer et al., 2014)
Sula variegata	*S. nebouxii*	nDNA	Sexual Selection	(Taylor et al., 2013)

Hippolais icterina	*H. polyglotta*		Hybrid Zone Movement	(Secondi et al., 2006)
Aegithalos fuliginosus	*A. bonvolati*	mtDNA		(Wang et al., 2014)
Plectrophenax hyperboreus	*P. nivalis*		Range Expansion	(Maley and Winker, 2010)
Passerina cyanea	*P. amoena*	mtDNA		(Carling and Brumfield, 2008)
Coereba flaveola (island populations)		mtDNA		(Bellemain et al., 2008)
Ammodramus caudacutus	*A. nelsoni*	nDNA		(Shriver et al., 2005)
Emberiza leucocephala	*E. citrinella*	mtDNA	Selective Sweep	(Irwin et al., 2009b)
Zonotrichia leucophrys	*Zonotrichia atricapilla*	mtDNA	Range Expansion	(Weckstein et al., 2001)
Garrulax chinensis chinensis	*G. c. lochmius*	mtDNA - nDNA	Range Expansion	(Wu et al., 2012)
Malurus melanocephalus cruentatus	*M. m. melanocephalus*	Plumage	Sexual Selection	(Baldassarre et al., 2014)
Luscinia megarhynchos	*L. luscinia*	nDNA		(Storchova et al., 2010)
Baeolophus inornatus	*B. ridgwayi*		Range Expansion	(Cicero, 2004)
Setophaga occidentalis	*S. townsendi*	mtDNA	Hybrid Zone Movement	(Krosby and Rohwer, 2009)
Setophaga coronata	*S. auduboni*	mtDNA	Migration (Adaptation)	(Toews et al., 2014)
Manacus vitellinus	*M. candei*	Plumage	Sexual Selection	(Parsons et al., 1993)
Foudia omissa	*F. madagascariensis*	mtDNA		(Warren et al., 2012)
Phyllastrephus debilis rabai	*P. debilis albigula*		Range Expansion	(Fuchs et al., 2011)
Thryothorus nigricapillus castaneus	*T. n. nigricapillus*	mtDNA	Sexual Selection	(Gonzalez et al., 2003)
Catharus ustulatus swainsoni	*C. u. ustulatus*	mtDNA	Migration (Arrival Time)	(Ruegg, 2008)

4.4 Conclusions and Outlook

There is an extensive amount of scientific literature on avian hybrid zones and consequent patterns of introgression. We identified 114 avian hybrid zones that have been described from a morphological or genetic perspective. Most avian hybrid zones have been classified as tension zones, assuming a balance between dispersal into the zone and decreased fitness of the hybrids. Some avian hybrid zones seem to fit the bounded hybrid superiority zone model, in which hybrids are more fit compared to the parental taxa in restricted areas. Moreover, it may be possible that a hybrid zone alternates between tension zone and hybrid superiority zone dynamics. Most hybrids zones are probably the outcome of secondary contact after an allopatric phase, but discriminating between primary and secondary contact zones is challenging.

Several striking patterns of introgression have been documented. Differential introgression patterns among several genomic classes, such as autosomal, mitochondrial and sex-linked loci, can be explained by Haldane's Rule and sex-biased dispersal. The generality of these proposed mechanisms remains to be determined. Asymmetric introgression can be the result of a numerous processes, such as simple demographic processes (e.g., range expansion), natural selection, and complex behaviours, including interspecific forced copulations and brood amalgamation.

Future research into avian hybrid zones and introgression is expected to rely on genomic data (Toews et al., 2016, Kraus and Wink, 2015, Jarvis, 2016). Whole-genome sequences will provide detailed information about patterns of divergence between hybridizing and diverging taxa and about patterns of variation across hybrid zones (Harrison and Larson, 2016). Genomic data will also lead to more detailed insights into the patterns of introgression across a hybrid zone, the genes involved in reproductive isolation and the evolutionary history and origin of the hybrid zone. Several avian hybrid zones have already been characterized using genomic resources (Ellegren et al., 2012, Poelstra et al., 2014) and many more are expected to follow in the near future. As already exclaimed by Kraus and Wink (2015): "avian genomics is fledging into the wild".

Chapter 5

Birds in a bush: toward an avian phylogenetic network

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Abstract

Reconstructing the avian tree of life has become one of the major goals in ornithology. The use of genomic tools seemed a promising approach to reach this goal, but, instead, phylogenetic analyses of large numbers of genes uncovered high levels of incongruence between the resulting gene trees. This incongruence can be caused by several biological processes, such as recombination, hybridization and rapid speciation (which can lead to incomplete lineage sorting). These processes directly or indirectly amount to deviations from tree-like patterns, thereby thwarting the use of phylogenetic trees. Phylogenetic networks provide an ideal tool to deal with these difficulties. We illustrate the usefulness of phylogenetic networks to capture the complexity and subtleties of diversification processes by discussing several recent genomic analyses of birds in general and the well-known radiation of Darwin's Finches. With the increasing amount of genomic data in avian phylogenetic studies, capturing the evolutionary history of a set of taxa in a phylogenetic tree will become increasingly difficult. Moreover, given the widespread occurrence of hybridization and the numerous adaptive radiations in birds, phylogenetic networks provide a powerful tool to display and analyse the evolutionary history of many bird groups. The genomic era might thus result in a paradigm shift in avian phylogenetics from trees to bushes.

5.1 Toward an Avian Phylogenetic Network

The most iconic drawing in evolutionary biology was scribbled around July 1837 in a notebook by Charles Darwin. The drawing depicts a crude evolutionary tree with the words "I think" above it. In *The Origin of Species*, he further developed this idea, which was already circulating in scientific circles in pre-Darwinian times (Archibald, 2009), into the metaphor of the tree of life (Darwin, 1859):

> "The affinities of all the beings of the same class have sometimes been represented by a great tree. […] As buds give rise by growth to fresh buds, and these if vigorous, branch out and overtop on all sides many a feebler branch, so by generation I believe it has been with the great Tree of Life, which fills with its dead and broken branches the crust of the earth, and covers the surface with its ever branching and beautiful ramifications."

Reconstructing the tree of life has become one of the major goals in evolutionary biology, but is the tree of life still viable in a phylogenetic context with high levels of interspecific gene exchange? Should we abandon the tree of life metaphor and turn to a network approach?

Until the 1970s evolutionary trees were largely based on the analysis of morphological characters. The use of molecular data in phylogenetics led to a revolution. The most influential methods were protein electrophoresis in the late 1960s and 1970s, RFLP (restriction fragment length polymorphism) analyses in the 1970s and 1980s, and PCR-mediated DNA sequencing in the 1990s (Avise, 2004, Kraus and Wink, 2015). At first, a few genes became reference markers. For instance, the gene that encodes the small subunit ribosomal RNA (SSU rRNA) was extensively used for phylogenetic analyses of microorganisms and led to the discovery of a third domain of life, the Archaea (Woese and Fox, 1977). But as more and more genes were sequenced and analysed, it became clear that different genes often result in discordant gene trees (Maddison, 1997, Pamilo and Nei, 1988).

The advent of multilocus data showed that the occurrence of phylogenetic incongruence (i.e. analyses of different genes resulting in discordant gene trees) is a common and widespread phenomenon (Rokas et al., 2003). Such incongruence can be caused by analytical shortcomings (Rokas et al., 2003, Davalos et al., 2012) or can be the result of biological processes, such as horizontal gene transfer, hybridization, incomplete lineage sorting and gene duplication (Maddison, 1997, Pamilo and Nei, 1988, Degnan and Rosenberg, 2009). Several methods have been developed to estimate a species tree from a collection of discordant gene trees (Delsuc et al., 2005, Degnan and Rosenberg, 2009, Liu et al., 2015). The construction of a species tree from several discordant gene trees is based on the assumption that the underlying evolutionary process is tree-like. But such phylogenetic trees are less suited to depict reticulate events, such as recombination, horizontal gene transfer and hybridization. In addition, some evolutionary mechanisms, such as incomplete lineage sorting, gene duplication and gene loss, result in incompatibilities that cannot be easily represented by a species tree. Phylogenetic networks provide an ideal tool to deal with these difficulties.

A phylogenetic network is defined as “any network in which taxa are presented by nodes and their evolutionary relationships are represented by edges” (Huson and Bryant, 2006). Phylogenetic networks can be used in two main ways: either to represent incompatibilities within and between data sets (implicit or abstract networks), or to represent the occurrence of reticulate events in the evolutionary history of a group of taxa (explicit networks). These networks are also called split networks and reticulate networks, respectively (Huson et al., 2010).

The tree of life houses several events of reticulate evolution. For example, the eukaryotic cell is probably the outcome of endosymbiosis between distantly related prokaryotes, also leading to the conversion of free-living bacteria into cell organelles, such as mitochrondria and chloroplasts (Margulis, 1993, Gupta and Golding, 1996). Furthermore, in the prokaryotic realm, horizontal gene transfer (i.e. the transfer of genetic material between distantly related lineages) is a common phenomenon (Andam and Gogarten, 2011a, Andam and Gogarten, 2011b, Gogarten et al., 2002, Gogarten and Townsend, 2005). Similarly, in eukaryotes, interspecific gene transfer by means of introgressive hybridization has been documented in numerous taxa (Anderson, 1949, Dowling and Secor, 1997, Mallet, 2005). Moreover, several plant (Rieseberg, 1997, Hegarty and Hiscock, 2005) and animal taxa (Mallet, 2007, Mavarez and Linares, 2008) are probably of hybrid origin. For example, the Italian Sparrow (*Passer italiae*) is probably a hybrid species between House Sparrow (*P. domesticus*) and Spanish Sparrow (*P. hispaniolensis*) (Elgvin et al., 2011, Hermansen et al., 2011). These examples indicate that many complex and successful lifeforms, such as the eukaryotic cell, could not be possible without reticulate evolution.

Apart from reticulate events, incompatibilities between gene trees can also be caused by other processes, such as incomplete lineage sorting. Several studies using multilocus data reported high levels of incomplete lineage sorting, hampering the estimation of species trees (e.g., Kutschera et al., 2014, Pollard et al., 2006, Willis et al., 2007, Barker et al., 2015). So, along with reticulation, incomplete lineage sorting results in a deviation from a tree-like depiction of evolutionary histories in a species tree. The tree of life might thus be better represented as the "net of life" (Martin, 1999, Doolittle, 1999, Kunin et al., 2005). Furthermore, in combination with the analysis of retrotransposons, phylogenetic networks can be used to quantify the degree of incomplete lineage sorting and to estimate the duration of the speciation process (Suh et al., 2015, Hallstrom and Janke, 2010).

From an analytical point of view, phylogenetic networks may also be an improvement on classical phylogenetic tree analyses. With the rapid growth of genomic data, sampling error (i.e. random error resulting from small sample sizes or short sequence reads) is becoming less of an issue, whereas systematic error (i.e. wrong assumptions in the underlying model of sequence evolution, leading to artefacts and biases in phylogenetic inference) is becoming increasingly important (Delsuc et al., 2005, Felsenstein, 2004). Unlike sampling error, systematic error cannot be avoided by increasing sequence length. Phylogenetic tree-building methods attempt to fit a tree to the data, even if a significant gap exists between the resulting tree and the data, possibly leading to phylogenetic artefacts (Steel, 2005).

Model-based split networks are able to deal with systematic error by adding extra parameters to the evolutionary model (Huson et al., 2010). Phylogenetic inference comprises two kinds of parameters: those describing the evolutionary model (e.g., substitution rates) and those describing the topology (e.g., branch lengths). Evolutionary models based on split networks contain extra topology-related parameters (allowing for reticulation) that may lead to a better fit to the data. Several studies have shown that split networks fit the data better than phylogenetic trees and that network analyses can uncover phylogenetic signals missed by tree-based methods (Esser et al., 2004, Kolaczkowski and Thornton, 2004).

Despite the usefulness of phylogenetic trees to depict reticulate events, to quantify incomplete lineage sorting and to deal with systematic error during phylogenetic inference, one main issue currently remains: there is as yet no standard way to interpret a phylogenetic network. What caused reticulations in a particular phylogenetic network? Hybridization? Incomplete lineage sorting? Analytical issues? Disentangling these processes and quantifying the relative contribution of each is challenging and requires the development of new tools and algorithms (Huson et al., 2010). This situation is similar to the mismatch between the rapid progress of next generation sequencing techniques and the relatively slow development of software to analyse the increasing amount of genomic data. The algorithms for estimating phylogenetic networks have not yet reached the complexity of phylogenetic tree methods, but this field of research is growing rapidly (e.g., Cardona et al., 2015, Huber et al., 2016, Solis-Lemus and Ane, 2016).

But what about birds? Are birds also entangled in this net of life? Based on the recent surge of avian genomic data (Kraus and Wink, 2015, Joseph and Buchanan, 2015), we argue that modern avian phylogenetics warrants a phylogenetic network approach to complement the classical (and still useful) concept the phylogenetic tree. We illustrate this with two examples: the contrasting results from two recent phylogenomic studies (Jarvis et al., 2014, Prum et al., 2015) and the outcome of a genomic perspective on the radiation of Darwin's Finches (Lamichhaney et al., 2015).

Joseph and Buchanan (2015) called it "a quantum leap in avian biology", the simultaneous publication of several papers (27 in eight journals) based on a genomic dataset of 48 bird species. One of these papers (Jarvis et al., 2014) presented a new and updated avian tree of life. A couple of months later, however, another avian tree (Prum et al., 2015) was published, with some contrasting results. For example, Jarvis et al. (2014) reported a well-supported clade consisting of the Hoatzin (*Opisthocomus*) as the sister group of plovers (*Charadrius*) and cranes (*Grus*), whereas Prum et al. (2015) identified the Hoatzin as a sister group of the core landbirds.

The contrasting results can be caused by analytical shortcomings (e.g., long branch attraction) or can be the result of biological processes, such as hybridization, incomplete lineage sorting, and gene duplication. The rapid diversification of modern birds after the mass extinction event about 66 million years ago (i.e. the K-Pg boundary) could lead to very short internal branches and high levels of incomplete lineage sorting (Rosenberg, 2013, Degnan and Rosenberg, 2009). Suh et al. (2015) quantified the amount of incomplete lineage sorting along the Neoaves phylogeny using presence/absence data for 2,118 retrotransposons. They uncovered discordant phylogenetic signals near the initial K-Pg radiation and at the base of two other radiations that gave rise to the core landbirds and the core waterbirds. They conclude that "as a consequence, their complex demographic history is more accurately represented as local networks within a species tree" (Figure 5.1). The complexity of this radiation was already apparent in a previous, although limited, analysis of retrotransposons (Hernandez-Lopez et al., 2013).

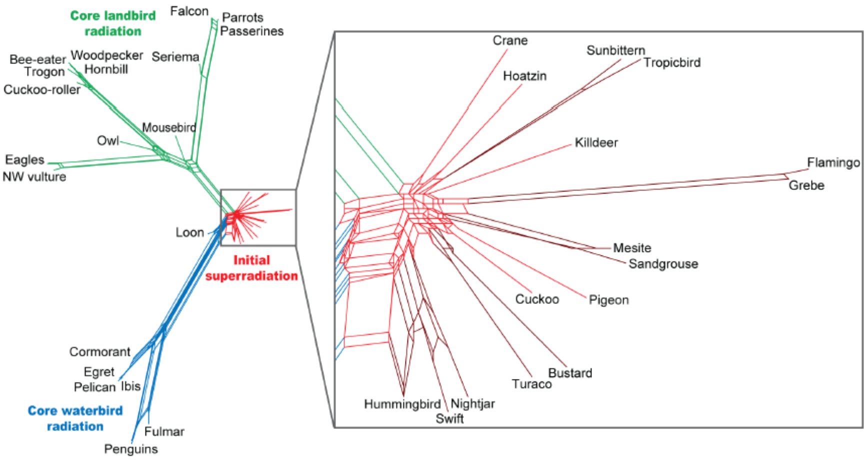


Figure 5.1 Phylogenetic network based on presence/absence data of 2118 retrotransposons. The initial radiation (red) at the base of the tree is depicted in greater detail. The other 2 radiations gave rise to the core landbirds (green) and the core waterbirds (blue). – Adapted from Suh et al. (2015).

This example shows the possible effect of incomplete lineage sorting during the diversification of modern birds, but we cannot rule out the possibility of introgressive hybridization, which can result in similar patterns (Maddison, 1997). More detailed analyses are necessary to disentangle the relative contributions of analytical issues, incomplete lineage sorting and hybridization during this rapid radiation. This analysis has been completed for the more recent adaptive radiation of Darwin's Finches on the Galapagos Islands (Almen et al., 2016). Using whole-genome re-sequencing data of 120 individuals, Lamichhaney et al. (2015) found evidence for extensive interspecific gene flow throughout the radiation. They constructed a phylogenetic network from autosomal genomic sequences to display the conflicting signals at the internal branches, caused by incomplete lineage sorting and hybridization (Figure 5.2).

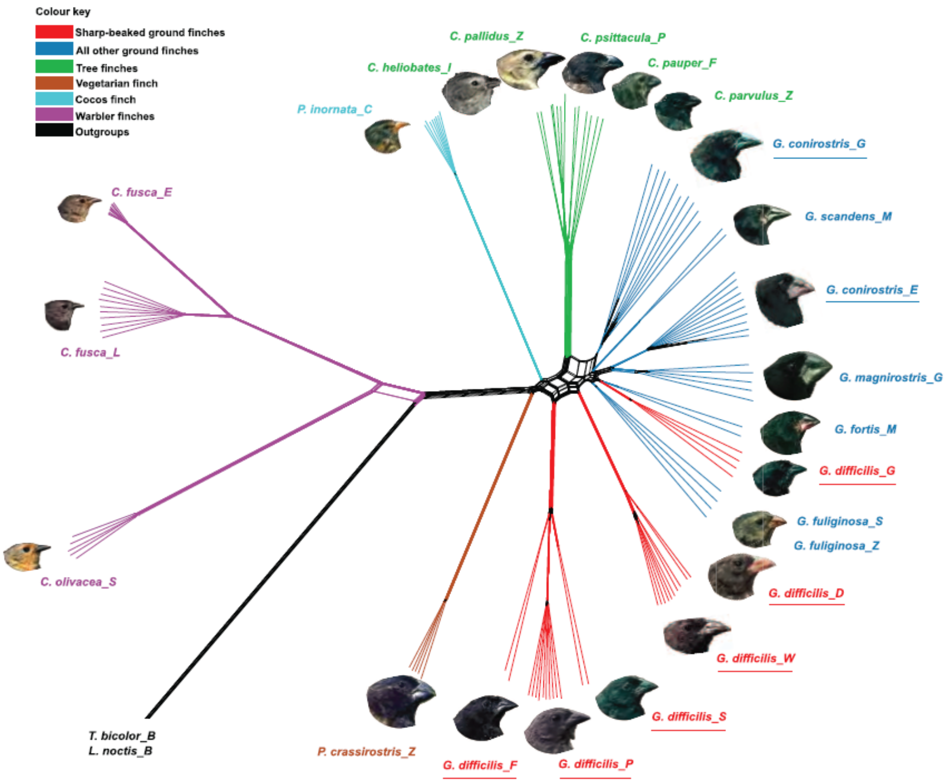


Figure 5.2 Phylogenetic network for the Darwin's finches based on whole genome resequencing data. – Adapted from Lamichhaney et al. (2015).

Other studies have also described complex evolutionary histories with high levels of gene flow and incomplete lineage sorting for several groups of closely related bird species (Lavretsky et al., 2014, Carling et al., 2010, Hung et al., 2012). The evolutionary histories of these bird groups have all been forced into a phylogenetic tree, whereas a phylogenetic network may have been a better option to capture the complexity and subtleties of the diversification processes. Traditionally, speciation has been viewed as the splitting of an ancestral population into two reproductively isolated species, a process that can easily be depicted as a bifurcation (Dobzhansky, 1937, Mayr, 1942). Recent genomic studies have shown, however, that speciation is a dynamic and complex process in which the incipient species often continue to exchange genes before they reach complete reproductive isolation (Nosil, 2008, Mallet et al., 2016, Pinho and Hey, 2010). With the increasing amount of genomic data in avian phylogenetic studies, capturing the evolutionary history of a set of taxa in a phylogenetic tree will become increasingly difficult. Given the widespread occurrence of hybridization (Chapter 3) and the numerous adaptive radiations (Jetz et al., 2012) in birds, phylogenetic networks will provide a powerful tool to display and analyse the evolutionary history of many bird groups. The genomic era might thus result in a paradigm shift in avian phylogenetics from trees to bushes.

PART II

GOOSE GENOMICS

Chapter 6

Hybridization in geese: a review

Ottenburghs, J., van Hooft, P., van Wieren, S.E., Ydenberg, R.C. & Prins, H.H.T. (2016) Hybridization in geese: a review. *Frontiers in Zoology*, 13:20.

Abstract

The high incidence of hybridization in waterfowl (ducks, geese and swans) makes this bird group an excellent study system to answer questions related to the evolution and maintenance of species boundaries. However, knowledge on waterfowl hybridization is biased towards ducks, with a large knowledge gap in geese. In this review, we assemble the available information on hybrid geese by focusing on three main themes: (1) incidence and frequency, (2) behavioural mechanisms leading to hybridization, and (3) hybrid fertility. Hybridization in geese is common on a species level, but rare on a per-individual level. An overview of the different behavioural mechanisms indicates that forced extra-pair copulations and interspecific nest parasitism can both lead to hybridization. Other sources of hybrids include hybridization in captivity and vagrant geese, which may both lead to a scarcity of conspecifics. The different mechanisms are not mutually exclusive and it is currently not possible to discriminate between the different mechanisms without quantitative data. Most hybrid geese are fertile; only in crosses between distantly related species do female hybrids become sterile. This fertility pattern, which is in line with Haldane's Rule, may facilitate interspecific gene flow between closely related species. The knowledge on hybrid geese should be used, in combination with the information available on hybridization in ducks, to study the process of avian speciation.

6.1. Introduction

Hybridization, the interbreeding of species, has always intrigued ornithologists. Ernst Mayr (1942) pointed out that "In birds, we have a fair amount of information, since some collectors, sensing their scarcity value, have specialized in the collecting of hybrids, and amateur observers have always been fascinated by them." The first attempt to compile the numerous scattered references and reports of avian hybrids was undertaken by Suchetet (1897). Later on, many more checklists and compilations of avian hybrids have been published (Cockrum, 1952, Meise, 1975, Gray, 1958, Grant and Grant, 1992, Panov, 1989, Ottenburghs et al., 2015, Mayr and Short, 1970, McCarthy, 2006). The incidence of hybridization varies among bird orders, with the Anseriformes (waterfowl: ducks, geese and swans) showing the highest propensity to hybridize. Over 60% of waterfowl species has hybridized with at least one other species and this figure increases to almost 77% when including captive hybrids (Ottenburghs et al., 2015).

The high incidence of hybridization in waterfowl makes this bird group an excellent study system to answer questions related to the origin and preservation of species. For example, how do waterfowl species remain distinct despite high levels of hybridization? Does hybridization lead to the exchange of genetic material (i.e. introgression) and if so, does this provide individuals with an adaptive advantage or disadvantage? Indeed, there are still many open questions in speciation and hybridization research that could be answered by studying hybridization in waterfowl (Abbott et al., 2013, Butlin et al., 2012). These questions, however, are not the focus of this review.

The knowledge on waterfowl hybridization is biased towards ducks, as illustrated by an extensive inventory of hybrid ducks (Gillham and Gillham, 1998), an analysis of hybrid duck fertility patterns (Tubaro and Lijtmaer, 2002) and several genetic studies documenting interspecific gene flow due to introgressive hybridization (e.g., Kraus et al., 2012, Peters et al., 2014a, Lavretsky et al., 2014). The knowledge of goose hybrids is clearly lagging behind. Several studies reported goose hybrids (Delnicki, 1974, Craven and Westemeier, 1979, Weckstein et al., 2002, Nijman et al., 2010) or provided a description of local records of hybrid geese (Bloomfield, 2004, Kampe-Persson and Lerner, 2007, Randler, 2008), but no study has been dedicated to the incidence of goose hybrids or their fertility. The differences in species discrimination and social structure between ducks and geese provide the opportunity to formulate and test research questions that will broaden our understanding on the origin and preservation of waterfowl species. For instance, how does sexual selection (as measured by the degree of sexual dimorphism) relate to the frequency of hybridization? Does hybridization accelerate of slow down the speciation process? Which behavioural and morphological characateristics determine conspecific or heterospecific mate choice? Is there strong selection against hybrids?

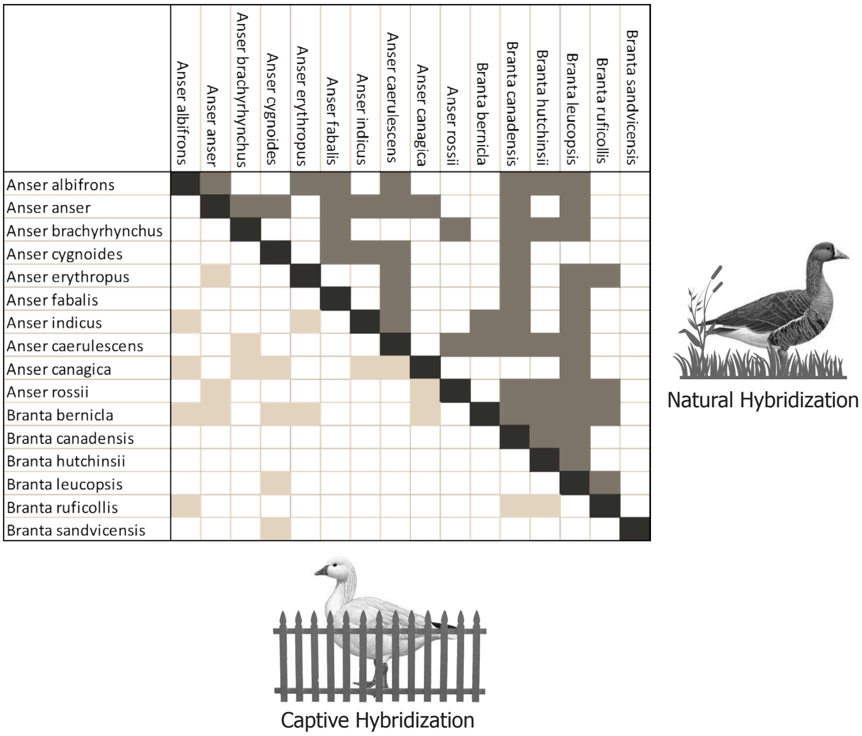
In this review, we address the knowledge gaps on hybrid geese by focusing on three main themes: (1) incidence and frequency, (2) behavioural mechanisms leading to hybridization, and (3) hybrid fertility.

6.2. Goose Taxonomy

Table 1.1 (see Chapter 1) gives an overview of the current taxonomic classification of the True Geese. We follow the International Ornithologists' Union (IOU) for species names (Gil and Donsker, 2013), with one exception. Even though IOU currently recognizes two species of Bean Goose (Taiga Bean Goose and Tundra Bean Goose), most reports on hybridization date from before this split into two species and hence, it is not possible to analyse these Bean Goose species separately.

6.3. Incidence and Frequency of Goose Hybrids

There is an important distinction between incidence and frequency of hybridization. Incidence is binary: a certain hybrid combination has been observed or not. Figure 6.1 gives an overview of 74 observed hybrid geese in nature and captivity, based on records retrieved from the Serge Dumont Hybrid Database (Dumont, 2014). The frequency of hybridization refers to the number of hybrid individuals in the wild. Because actual numbers of hybrids are mostly not included in bird counts and some crosses are very hard to identify (Randler, 2004), it is nearly impossible to get an accurate estimate of the number of hybrids for certain combinations of species. However, two surveys in Great Britain monitored the frequency of hybrid geese in 1991 and 2000 when occurrence of the most common hybrid (Canada Goose x Greylag Goose) was quantified. These hybrids represent less than one per cent of the British population of Canada Geese and Greylag Geese (0.33% in 1991 and 0.11% in 2000) (Delany, 1992, Rowell et al., 2004), falling in line with previous estimates from other bird groups (Parmenter and Byers, 1991, Gillham and Gillham, 1998, Curson et al., 2010, Harrap and Quinn, 2010).



	Anser albifrons	Anser anser	Anser brachyrhynchus	Anser cygnoides	Anser erythropus	Anser fabalis	Anser indicus	Anser caerulescens	Anser canagica	Anser rossii	Branta bernicla	Branta canadensis	Branta hutchinsii	Branta leucopsis	Branta ruficollis	Branta sandvicensis
Anser albifrons	■	▨			▨	▨		▨				▨	▨	▨		
Anser anser		■	▨	▨		▨	▨	▨	▨			▨		▨		
Anser brachyrhynchus			■			▨				▨		▨	▨	▨		
Anser cygnoides				■		▨	▨	▨				▨				
Anser erythropus		░			■			▨				▨		▨	▨	
Anser fabalis						■		▨				▨		▨		
Anser indicus	░				░		■	▨			▨	▨		▨		
Anser caerulescens			░					■		▨	▨	▨	▨	▨		
Anser canagica	░		░	░			░	░	■					▨		
Anser rossii		░							░	■		▨	▨	▨	▨	
Branta bernicla	░	░		░	░				░		■	▨	▨	▨	▨	
Branta canadensis												■	▨	▨		
Branta hutchinsii													■	▨		
Branta leucopsis				░										■	▨	
Branta ruficollis	░											░	░		■	
Branta sandvicensis				░												■

Figure 6.1. Overview of incidence of hybridization in geese. Hybridization in nature is depicted above the diagonal, whereas hybridization in captivity below the diagonal. Species that hybridized both in nature and in captivity are included only in the former category.

Several European studies have compiled the occurrence of hybrid geese based on data from a variety of sources, such as regional and local bird magazines or personal observations (Table 6.1). In all studies, hybrids between Canada Goose and Greylag Goose were most numerous, while other hybrid geese were limited to a handful of individuals (Kampe-Persson and Lerner, 2007, Randler, 2008, Rowell et al., 2004). It seems that hybridization in geese is common on a species-level (Figure 6.1), but rare on a per-individual level (Table 6.2). Although hybrids are rare in populations, a few hybrids can provide a bridge for interspecific gene flow (Mallet, 2005), which can have important evolutionary consequences, such as adaptive introgression (Hedrick, 2013).

Table 6.1. Frequency of hybrid geese recorded in three countries: Germany (Randler, 2000), Great Britain (Rowell et al., 2004) and Sweden (Kampe-Persson and Lerner, 2007).

Hybrid	**Germany**	**Great Britain**	**Sweden**
Barnacle Goose x Canada Goose	6	8	33
Barnacle Goose x Lesser White-fronted Goose	1		15
Barnacle Goose x Greylag Goose			4
Barnacle Goose x Bar-headed Goose	5	1	1
Barnacle Goose x Emperor Goose		5	
Barnacle Goose x Greater White-fronted Goose	3		1
Barnacle Goose x Red-breasted Goose	1		1
Barnacle Goose x Ross' Goose	1		
Barnacle Goose x Snow Goose		2	
Lesser x Greater White-fronted Goose			2
Greylag Goose x Canada Goose	140	88	226
Greylag Goose x Bar-headed Goose	6	6	2
Greylag Goose x Greater White-fronted Goose		12	1
Greylag Goose x Snow Goose		20	
Greylag Goose x Swan Goose	38	57	1
Canada Goose x Bar-headed Goose	12	1	1
Canada Goose x Greater White-fronted Goose			6
Canada Goose x Swan Goose	3	4	
Bar-headed Goose x Emperor Goose		1	
Swan Goose x Bar-headed Goose	12		

6.4. Origin of Goose Hybrids

Several behavioural mechanisms have been called upon to explain the production of hybrid offspring in birds (Wirtz, 1999, Randler, 2002, Randler, 2006). Here, we discuss four mechanisms that are relevant for the occurrence of goose hybrids, namely (1) nest parasitism, (2) extra-pair copulations, (3) rarity of conspecifics, and (4) captive birds.

6.4.1 Nest Parasitism

Nest parasitism and brood amalgamation occur commonly in waterfowl, both within and among species (Eadie et al., 1988, Rohwer and Freeman, 1989, Beauchamp, 1997). Intraspecific nest parasitism has been documented for several goose species (Table 6.2), but only three goose species are known to show interspecific nest parasitism, namely Greylag Goose, Snow Goose and Canada Goose (Beauchamp, 1998, Kampe-Persson and Lerner, 2007). Interspecific nest parasitism could facilitate hybridization because hatching by a heterospecific foster parent might lead to sexual imprinting on the foster parent's species and this may in turn lead to interspecific mate choice in the future (Figure 6.2). The plausibility of this scenario has been assessed experimentally by means of cross-fostering experiments: Fabricius (1991) placed eggs of Greylag Geese in the nest of Canada Geese. The young Greylag Geese followed their foster parents to their wintering grounds. On return, all females (16) paired with Greylag Geese, whereas 5 out of 19 males paired with Canada Geese. Furthermore, some Greylag Goose males that lost a partner remated with a female Canada Goose, showing that these males were sexually imprinted on this species.

Some goose species adopt conspecific young (Larsson et al., 1995, Williams, 1994, Kalmbach, 2006, Choudhury et al., 1993, Zicus, 1981). Whether geese also adopt heterospecific goslings and if this adoption can affect sexual imprinting and future mate choice is unknown. Heterospecific adoption has been documented between several distantly related bird species, but seems to be a rare phenomenon (Shy, 1982).

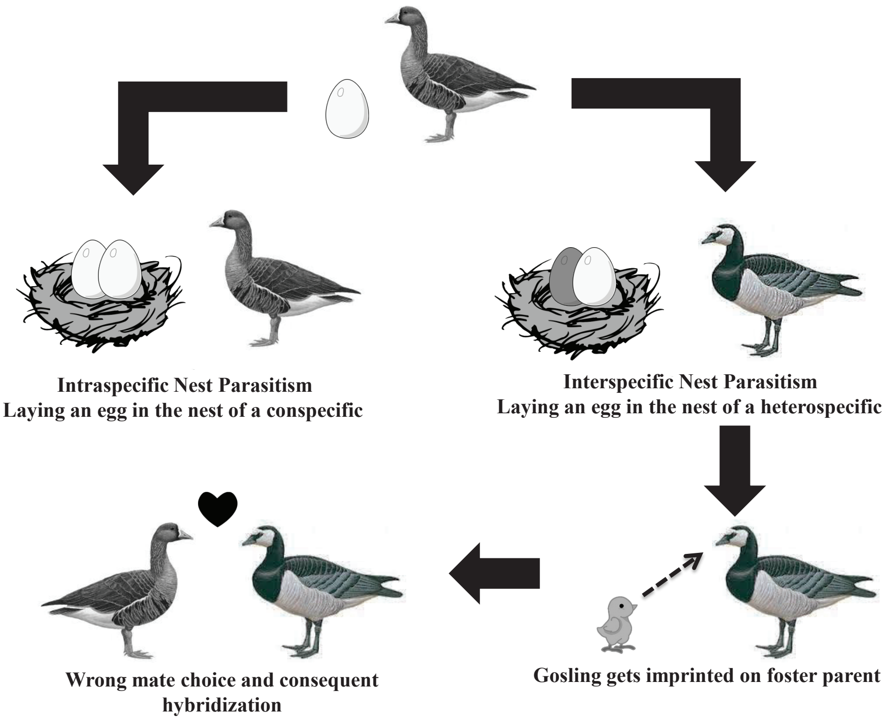


Figure 6.2. Graphical representation showing how interspecific nest parasitism can lead to hybridization

Table 6.2. Occurrence of intra- and interspecific nestparastism and extra-pair copulations in all goose species.

Species	**Nest Parasitism**		**Extra-pair Copulations**
	Intraspecific	**Interspecific**	
Swan Goose			
Bean Goose			
Pink-footed Goose			
Greater White-fronted Goose			[18]
Lesser White-fronted Goose			
Bar-headed Goose	[1]		
Greylag Goose	[2]	[16]	
Snow Goose	[3-6]	[17]	[4,19]
Ross' Goose	[7]		[19]
Emperor Goose	[8]		
Hawaii Goose			
Canada Goose	[9-10]	[17]	[20]
Barnacle Goose	[11-13]		
Brent Goose	[14-15]		[21]
Red-breasted Goose			

References: 1. Weigmann and Lamprecht (1991), 2. Cramp and Simmons (1977), 3. Lank et al. (1989a), 4. Lank et al. (1989b), 5. Lank et al. (1990), 6. Syroechkovsky (1979), 7. Bellrose and Kortright (1976), 8. Eisenhauer and Kirkpatrick (1977), 9. Prevett et al. (1972), 10. Seddon and Nudds (1994), 11. Larsson et al. (1995), 12. Anderholm et al. (2009), 13. Choudhury et al. (1993), 14. Bregnballe and Madsen (1990), 15. Spaans et al. (1993), 16. Kampe-Persson and Lerner (2007), 17. Beauchamp (1998), 18. Ely (1989), 19. Dunn et al. (1999), 20. Moore et al. (2012), 21. Welsh and Sedinger (1990)

6.4.2 Extra-pair copulations

Forced extra-pair copulations (often called "rapes") have been reported in several species of waterfowl (Mckinney et al., 1983). Trivers (1972) suggested that such extra-pair copulations could be functional; he noted that "a mixed strategy will be the optimal male course – to help a single female raise young, while not passing up opportunities to mate with other females whom he will not aid." Males of several goose species engage in forced extra-pair copulations, such as Greater White-fronted Goose (Ely, 1989), Brent Goose (Welsh and Sedinger, 1990) and Canada Goose (Moore et al., 2012). But this behaviour has been studied most extensively in Snow Goose and Ross' Goose (Dunn et al., 1999, Mineau and Cooke, 1979, Lank et al., 1989b). In the Canadian Karrak Lake Colony, Dunn et al. (1999) observed that among successful copulations, 33% and 38% were extra-pair in Ross' and Snow Geese, respectively. Despite this high precentage of extrapair copulations, only 2-5% of the goslings had another father than the male guarding the nest. A similar low percentage of extra-pair paternity (2-4%) was also reported for Snow Geese in northern Manitoba, Canada (Lank et al., 1989b). Based on these low ferilization percentages, forced extra-pair copulations appear to be a relatively inefficient reproductive tactic for males of these goose species. However, offspring resulting from successful extra-pair copulations do provide a fitness benefit to males.

Extra-pair copulations can lead to hybridization when males copulate with females of another species. This has been observed for ducks: for instance, Seymour (1990) reported three occasions of an extra-pair copulation attempt by a male Mallard (*Anas platyrhynchos*) on a female Black Duck (*Anas rubripes*). However, interspecific extra-pair copulations have not been documented in geese. This can be due to the limited number of behavioral studies of geese during the period when copulations are most likely, and may also reflect differences in species discrimination and social structure between ducks and geese (Mckinney et al., 1983). Male ducks often seem unable or indifferent to discriminate between females of different species (which look very similar) as many studies report male ducks displaying to heterospecific females (Randler, 2002, Bossema and Kruijt, 1982, Brodsky et al., 1988). The social structure of geese, with long-term pairbonds and nest guarding by males, limits the opportunties for males to seek extra-pair copulations (Owen, 1980). Although interspecfic extra-pair copulations can potentially result in hybrid offspring, this behavioural mechanism seems of minor importance in the origin of hybrid geese, because of its low frequency and the low fertilization rate of such extra-pair copulations. This conclusion is in line with the study by Randler (2005), who showed that "interspecific brood amalgamation has a stronger impact on natural hybridization in wildfowl than forced extra-pair copulations."

6.4.3 Scarcity of conspecifics

Hubbs' Principle or the Desperation Hypothesis states that the rarer species is more likely to mate with heterospecifics (Hubbs, 1955). There are several situations in which individual birds can be confronted with a scarcity of conspecifics, such as range expansion, vagrant birds or the release/escape of captive birds in a non-native environment. With regard to geese, range expansion should include an expansion of the wintering grounds, where mate choice occurs (Rohwer and Anderson, 1988). Some birds will "make the best out of a bad job" and pair with a heterospecific mate: hybridizing with a closely related species may be a better solution than remaining unpaired (Baker, 1996, Randler, 2006). For instance, Indigo Buntings (*Passerina cyanea*) and Lazuli Buntings (*Passerina amoena*) switched to heterospecifics when no conspecific mates were available (Baker, 1996). Another good example of the Desperation Hypothesis concerns two duck species on the Falkland Islands, where Speckled Teals (*Anas flavirostris*) outnumber Yellow-billed Pintails (*Anas georgica*) about ten to one. This numerical imbalance leads to hybridization (McCracken and Wilson, 2011). The Desperation Hypothesis is not restricted to natural situations, in captivity birds are often confronted with a scarcity of conspecifics and might choose to mate with the available heterospecifics.

6.4.4 Captive birds

Cockrum (1952) already noted that "If hybrids resulting from birds in captivity were listed, the list would be much larger, especially among ducks and geese." Indeed, numerous hybrids have been produced in captivity (Ottenburghs et al., 2015). The occurrence of hybridization in captivity can be explained by the mechanisms discussed above, namely extra-pair copulations, nest parasitism and scarcity of conspecifics. When these hybrids escape, they can be mistakenly reported as wild hybrids. However, it may be possible to deduce the captive origin of hybrids when one of the parent species is not native by examining the range of occurrence. Table 6.1 shows that many hybrid geese probably have a captive origin; for instance, some of the most common hybrids in Europe are between Greylag Goose and two introduced species, Canada Goose and Swan Goose. However, there is also the possibility that vagrant geese enter the range of other species. For example, North American Snow Geese are occasionally observed in Europe during migration (Bruun, 1971) and hybrids between Snow Goose and several European species have been reported (McCarthy, 2006).

Randler (2000) introduced the "captivity effect" to account for the high rates of *Anser* hybrids in released populations. He argued that domestication of Greylag Goose and Swan Goose has resulted in genetic impoverishment and unnatural behaviour, leading to a relatively strong tendency for hybridization. For example, in Greylag Geese, the frequency of hybrids was higher in naturalised compared to natural populations (Sibley, 1994, Kampe-Persson and Lerner, 2007, Randler, 2000). The effects of captivity on hybridization should thus be taken into account.

6.5. Fertility of Goose Hybrids

In *The Origin of Species*, Darwin (1859) discussed the fertility of hybrids between two domesticated goose species, the Greylag Goose and the Swan Goose:

> "The hybrids from the common and Chinese geese (*A. cygnoides*), species which are so different that they are generally ranked in distinct genera, have often bred in this country with either pure parent, and in one single instance they have bred inter se. This was effected by Mr Eyton, who raised two hybrids from the same parents but from different hatches; and from these two birds he raised no less than eight hybrids (grandchildren of the pure geese) from one nest. In India, however, these cross-bred geese must be far more fertile; for I am assured by two eminently capable judges, namely Mr Blyth and Capt. Hutton, that whole flocks of these crossed geese are kept in various parts of the country; and as they are kept for profit, where neither pure parent-species exists, they must certainly be highly fertile."

Later, he repeated the experiment of Mr. Eyton by crossing "a brother and sister hybrid from the same hatch" that he received from Rev. Goodacre (Darwin, 1880). He only managed to rear five hybrids (several eggs did not hatch or remained unfertilized), but he was still startled by "the fact that these two species of geese [are] breeding so freely together." He attributed the fertility of these hybrids to the long history of goose domestication. We now know that, irrespective of domestication, the potential for hybridization is lost slowly on an evolutionary timescale in birds (Prager and Wilson, 1975) and that many bird species are capable of producing fertile hybrids (Price and Bouvier, 2002).

The evolution of hybrid sterility and inviability (both caused by postzygotic incompatibilities) has been studied in *Drosophila* (Coyne and Orr, 1989), frogs (Sasa et al., 1998), butterflies (Presgraves, 2002) and birds (Price and Bouvier, 2002). These studies showed an increase of postzygotic isolation between species with divergence time. Furthermore, the evolution of postzygotic incompatibility follows Haldane's Rule

(1922), which states that "when in the F_1 offspring of two different animal races one sex is absent, rare, or sterile, that sex is the heterozygous [or heterogametic] sex". In birds, where sex is determined by a ZZ/ZW system, females are the heterogametic sex and hybrid females are thus expected to show greater fitness reductions compared to male hybrids. This expectation has been confirmed for birds in general (Price and Bouvier, 2002), but also for specific bird groups, including ducks (Tubaro and Lijtmaer, 2002), galliform birds (Arrieta et al., 2013) and pigeons and doves (Lijtmaer et al., 2003).

One of the possible mechanisms that has been invoked to explain Haldane's rule is dominance theory, which is based on the Dobzhansky-Muller incompatibility model (Wu and Ting, 2004, Presgraves, 2010). Dominance theory states that hybrid sterility and inviability are the outcome of the interaction of two (or more) genes that have developed incompatible alleles in allopatry. If these alleles are recessive and located on the Z-chromosome, their effect will be much larger in female birds because this sex lacks another Z-chromosome that could hold a dominant version of the incompatible allele, which would nullify the negative effect of the recessive one. Moreover, it has been suggested that the Z-chromosome plays a disproportionately large role in the development of intrinsic incompatibilities (Presgraves, 2008). Several lines of evidence support this "Large Z-effect." First, Z-linked genes evolve faster compared to autosomal loci ("Faster Z-effect"), thereby speeding up the accumulation of incompatible alleles on this sex chromosome (Ellegren, 2009, Storchova et al., 2010). Second, if genes involved in premating and postzygotic isolation both occur on the Z-chromosome and thus become physically linked, it is expected that this facilitates the evolution of isolation barriers by means of reinforcement (Hall and Kirkpatrick, 2006). This situation has been described for *Ficedula* flycatchers, where genes for female preference and low hybrid fitness are located on the Z-chromosome (Saetre et al., 2003, Backström et al., 2010).

We tested whether geese also conform to Haldane's Rule. We obtained cytochrome b sequences from GENBANK and calculated genetic distances between taxa using the Maximum Composite Likelihood model with Gamma Distribution in MEGA6 (Tamura et al., 2013). Reports on hybrid goose fertility were collected from the Handbook of Avian Hybrids of the World (McCarthy, 2006). We performed a logistic regression in SPSS (version 19.0) with hybrid fertility as dependent variable (0 = both sexes fertile, 1 = only males fertile) and genetic distance as independent variable. To our knowledge, there are no reports of goose hybrids where only the females are fertile.

At high genetic distances, for most species only male hybrids are fertile (Figure 6.3, ß = 53.425, SE = 25.485, *z-value* = 2.096, *p* = 0.0361), a pattern that is consistent with Haldane's Rule. Two species pairs deviate from the expected pattern: only male hybrids between the congeneric Greater White-fronted Goose and Swan Goose are fertile and both sexes are fertile when crossing the more distantly related Canada Goose and Greater White-fronted Goose. However, a more detailed analysis is necessary to fully understand the evolution of postzygotic incompatibilities in geese. For example, Lijtmaer et al. (2003) studied postzygotic isolation in pigeons and doves based on records of old interspecific breeding experiments (Whitman and Riddle, 1919), which included data on the number of unhatched eggs and the sex ratio of clutches. Such analyses provide insights, not only into the fertility of hybrids, but also into the fertility and viability of backcrosses. For instance, Arrieta et al. (2013) showed that hybrid inviability was higher in F_2 compared to F_1 hybrids in galliform birds, indicating that interspecific gene flow may be hampered due to inviable F_2 hybrids. For geese, the fertility of male birds at high genetic distances suggests the possibility of interspecific gene flow between distantly related species (e.g., Greylag Goose and Canada Goose), but if consequent backcrosses are sterile or not viable, then the possibility of interspecific gene flow is greatly reduced. On the other hand, the fertility of hybrids at low genetic distances (e.g., Greater White-fronted Goose and Greylag Goose) provides the opportunity of interspecific gene flow between closely related species. For example, Leafloor et al. (2013) reported gene flow between Canada Goose and Cackling Goose across an arctic hybrid zone.

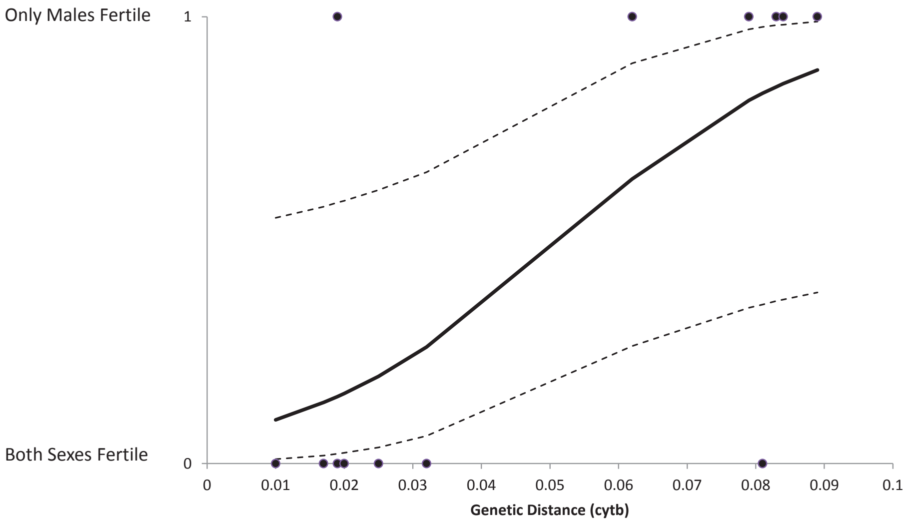


Figure 6.3. Fertility of goose hybrids at different genetic distances (based on cytochrome b sequences). At high genetic distances only male hybrids are fertile, a pattern in accordance with Haldane's Rule

6.6. Conclusions

Hybridization in geese is common on a species-level, but rare on a per-individual level. The origin of the occasional hybrids is difficult to determine. An overview of the different mechanisms shows that, in theory, interspecific nest parasitism or extra-pair forced copulations could lead to hybridization. Other sources of hybrids include a scarcity of conspecifics, hybridization in captivity and vagrant geese. The different mechanisms are not mutually exclusive, for instance, certain hybrids might be the result of extra-pair copulations in captivity. Currently, it is not possible to discriminate between the different mechanisms without quantitative data. To unravel the relative importance of these mechanisms, field data should be collected and experiments could be conducted in captivity. For example, the frequency of interspecific nest parasitism and extra-pair copulations may be documented in mixed breeding colonies. The occurrence of possible hybrids (which can be identified by means of genetic tests) in such colonies can then be related to the frequency of these behaviours. In captivity, experiments can be set up to observe how different goose species react to a scarcity of conspecifics and the availability of diverse heterospecifics. Most goose hybrids are fertile; only at high genetic distances do female hybrids become sterile. This fertility pattern provides the opportunity for interspecific gene flow between closely related species.

The overview of hybridization in geese presented here can be used, in combination with the knowledge available on duck hybrids, to study the process of avian speciation. Moreover, the differences in species discrimination and social structure between ducks and geese provide the opportunity to formulate and test research questions that will broaden our understanding on the origin and preservation of species.

Chapter 7

A tree of geese: a phylogenomic perspective on the evolutionary history of true geese

Ottenburghs, J., Megens, H.-J., Kraus, R.H.S., Madsen, O., van Hooft, P., van Wieren, S.E., Crooijmans, R.P.M.A., Ydenberg, R.C., Groenen, M.A.M. & Prins, H.H.T. (2016). A Tree of Geese: A Phylogenomic Perspective on the Evolutionary History of True Geese. *Molecular Phylogenetics and Evolution*. 101:303-313

Abstract

Phylogenetic incongruence can be caused by analytical shortcomings or can be the result of biological processes, such as hybridization, incomplete lineage sorting and gene duplication. Differentiation between these causes of incongruence is essential to unravel complex speciation and diversification events. The phylogeny of the True Geese (tribe Anserini, Anatidae, Anseriformes) was, until now, contentious, i.e. the phylogenetic relationships and the timing of divergence between the different goose species could not be fully resolved. We sequenced nineteen goose genomes (representing seventeen species of which three subspecies of the Brent Goose, *Branta bernicla*) and used an exon-based phylogenomic approach (41,736 exons, representing 5,887 genes) to unravel the evolutionary history of this bird group. We thereby provide general guidance on the combination of whole genome evolutionary analyses and analytical tools for such cases where previous attempts to resolve the phylogenetic history of several taxa could not be unravelled. Identical topologies were obtained using either a concatenation (based upon an alignment of 6,630,626 base pairs) or a coalescent-based consensus method. Two major lineages, corresponding to the genera *Anser* and *Branta*, were strongly supported. Within the *Branta* lineage, the White-cheeked Geese form a well-supported sub-lineage that is sister to the Red-breasted Goose (*Branta ruficollis*). In addition, two main clades of *Anser* species could be identified, the White Geese and the Grey Geese. The results from the consensus method suggest that the diversification of the genus *Anser* is heavily influenced by rapid speciation and by hybridization, which may explain the failure of previous studies to resolve the phylogenetic relationships within this genus. The majority of speciation events took place in the late Pliocene and early Pleistocene (between 4 and 2 million years ago), conceivably driven by a global cooling trend that led to the establishment of a circumpolar tundra belt and the emergence of temperate grasslands. Our approach will be a fruitful strategy for resolving many other complex evolutionary histories at the level of genera, species, and subspecies.

7.1. Introduction

Incongruence between phylogenies generated from different sets of genetic and phenotypic data poses a significant challenge in evolutionary biology. Such incongruence can be caused by analytical shortcomings (Davalos et al., 2012, Rokas et al., 2003), such as issues with limited taxon sampling, unmet assumptions in the modelling of sequence evolution, and the choice of different optimality criteria (Yang et al., 1994, Rokas et al., 2003, Graybeal, 1998). Phylogenetic incongruence can also be generated by biological processes, such as hybridization, incomplete lineage sorting and gene duplication (Maddison, 1997, Degnan and Rosenberg, 2009, Pamilo and Nei, 1988). Differentiation between these causes of incongruence is essential to unravel complex speciation and diversification events.

Although phylogenetic incongruence compromises the estimation of species trees, it can be a virtue for the evolutionary biologist, who attempts to better understand complex speciation events that are often obscured by rapid diversification and hybridization (Degnan and Rosenberg, 2009, Toews and Brelsford, 2012). Genomes are mosaics of many different gene histories (Ellegren et al., 2012, Payseur, 2010, Maddison, 1997) that can be compared to deduce the species tree, while also gaining more insights into intricate events of the speciation process, such as hybridization episodes or bursts of rapid diversification (Innan and Watanabe, 2006). In addition, conflicting gene histories can also be used to infer population sizes and divergence times (Rannala and Yang, 2003, Wall, 2003). Analysing phylogenetic incongruence from a genomic perspective of diverse gene histories will lead to a more complete picture of the speciation process.

The occurrence of phylogenetic incongruence is a common and widespread phenomenon: analyses of different genes often lead to discordant gene trees (Rokas et al., 2003). Consequently, several methods have been developed to estimate the species tree from a collection of contrasting gene trees (Delsuc et al., 2005, Degnan and Rosenberg, 2009). In concatenation (or supermatrix) methods all sampled genes are concatenated and analysed as a single "supergene" (de Queiroz and Gatesy, 2007). Supertree methods, on the other hand, involve separate analyses of the sampled genes and subsequent integration of the resulting gene trees into a species tree (Bryant, 2003). Certain supertree methods incorporate the multispecies coalescent model to estimate the species tree from a set of heterogeneous gene trees (Knowles, 2009)a. The multispecies coalescent model extends the classical coalescent (Kingman, 1980) to multiple populations and describes gene trees as independent random variables generated from the coalescence process occurring along lineages of the species tree (Liu et al., 2015). A number of coalescent-based methods have been developed to estimate the species tree from multigene sequence data, including Bayesian (e.g., BUCKy, Ane et al., 2007, BEAST, Drummond et al., 2012) and likelihood approaches (e.g., STEM, Kubatko et al., 2009, STELLS, Wu, 2012). Coalescent-based methods have been successfully implemented to disentangle the complex evolutionary history of several closely related bird species (Lavretsky et al., 2014, Carling et al., 2010, Hung et al., 2012).

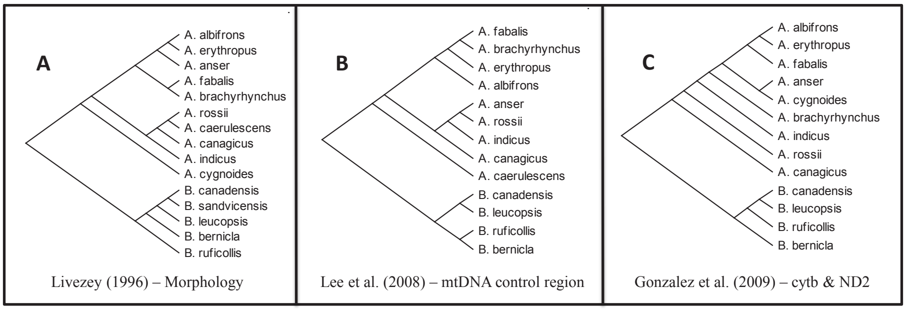


Figure 7.1 Phylogenetic trees for the True Geese based on morphology or mitochondrial markers. (A) Morphology (Livezey, 1996). (B) Mitochondrial control region (Lee et al., 2008). (C) Cytochrome b and NADH hydrogenase 2 (Gonzalez et al., 2009).

The failure to unravel the phylogenetic relationships between goose species of the tribe Anserini (commonly referred to as True Geese, see Table 1.1 in Chapter 1 for a taxonomic overview of this tribe) can be attributed to high levels of phylogenetic incongruence (Ruokonen et al., 2000). Although there have been several studies delving into the phylogeography of particular goose species (Ruokonen et al., 2005, Ruokonen et al., 2008, Scribner et al., 2003, Paxinos et al., 2002, Quinn, 1992, Jonker et al., 2013, Humphries et al., 2009, Volkovsky et al., 2013), the phylogenetic relationships between most species remained unclear. Until now, species-level phylogenies were based either on morphology (Livezey, 1996), or mitochondrial markers (Ruokonen et al., 2000, Lee et al., 2008, Donne-Gousse et al., 2002, Gonzalez et al., 2009). These approaches resulted in contradicting topologies and different mitochondrial markers led to different phylogenetic reconstructions (Figure 7.1). Phylogenetic incongruence is particularly apparent in the genus *Anser*. Although phylogenetic incongruence can be caused by hybridization and/or incomplete lineage sorting, all previous studies on goose phylogeny were based on mitochondrial DNA (mtDNA), which suggests analytical issues as the most likely cause of these incongruences. One example of the discrepancies among previous studies involves the phylogenetic position of the Lesser White-fronted Goose (*Anser erythropus*). Morphological analyses (Livezey, 1996) and some mitochondrial markers (cytb and ND2, Donne-Gousse et al., 2002, Gonzalez et al., 2009) report a sister relationship with the Greater White-fronted Goose (*Anser albifrons*), whereas studies using the mitochondrial control region (Lee et al., 2008, Ruokonen et al., 2000) place the Lesser White-fronted Goose in a clade with Taiga Bean Goose (*Anser fabalis*) and Pink-footed Goose (*Anser brachyrhynchus*).

In accordance with the lack of a well-supported phylogeny, the timing of origin of the extant goose species is unknown too. Fossil evidence indicates that geese were present during the Miocene and Pliocene (Brodkorb, 1964) and several phylogeographic studies reported Pleistocene origins of certain goose subspecies (Shields, 1990, Van Wagner and Baker, 1990, Avise et al., 1992). Moreover, a mtDNA study of the genus *Anser* dated speciation events to the late Pliocene and early Pleistocene (Ruokonen et al., 2000). Based on the available evidence, we hypothesize that the diversification of modern goose species was initiated in the Pliocene, accompanied by further diversification into distinct subspecies during the Pleistocene. Recent developments in divergence timing methods, that integrate both genomic data and fossil calibration points, can be applied to test this hypothesis (Parham et al., 2012).

The objectives of this study were threefold: (1) to unravel the phylogenetic relationships within the Anserini tribe using phylogenomic tools for species tree estimation, (2) to assess the timing of divergence for the extant goose species by means of genomic and fossil data and (3) discuss these findings in a framework of ecological, biogeographic and climatic events.

7.2. Materials and Methods

7.2.1 Sample Collection and DNA Extraction

We collected blood samples from 19 goose (sub)species (Table 1.1, see Table S7.1 for details). Genomic DNA was isolated from the blood samples using the Qiagen Gentra kit (Qiagen Inc.). Quality and quantity of the DNA was measured using the Qubit (Invitrogen, Life Technologies). Sequence libraries were made according to Illumina protocols and sequenced paired-end (100bp) on the Hiseq2500 (Illumina Inc.).

7.2.2 Processing Sequences

Paired-end reads (100 bp) were mapped to the Mallard (*Anas platyrhynchos*) genome, version 73 (Huang et al., 2013) using SMALT (http://www.sanger.ac.uk/resources/software/smalt/). Previous research indicated that SMALT is appropriate for mapping paired-end reads when the reference genome is distantly related to the sampled species (Frantz et al., 2013). Furthermore, preliminary analyses showed that this software produces the best results compared to other mapping software (Figure S7.1). SMALT uses a hash index of short words, sampled at equidistant steps along the reference genome. We applied default settings (word length = 13, spacing of hashed words = 6). Over 99% of the reads were mapped successfully in all samples, but to decrease the incidence of off-site mapping we only accepted properly mapped paired reads, resulting in mapping rates between 63% and 78% (Table S7.2). From the resulting BAM files duplicate sequences were excluded using SAMtools-dedup and realigned with IndelRealigner in GATK 2.6. Variant sites were called by means of the UnifiedGenotyper in GATK 2.6 with a minimum base quality of 20 and a heterozygosity value of 0.01 (this value has proven to be suitable for variant calling after mapping to a distantly related reference genome, see Frantz, 2015). The genomic positions of exonic sequences that were one-to-one orthologous between the Mallard and other bird species (Chicken, Turkey, Flycatcher and Zebra finch) were retrieved from the ENSEMBL data base. These genomic positions were used to extract the sequences from the different goose genomes. A final set of 41,736 unique exons (representing 5,887 genes) were concatenated, resulting in an alignment of 6,630,626 base pairs (bp).

Paired-end reads (100 bp) were also mapped to the Swan Goose (*Anser cygnoides*) genome (Lu et al., 2015) using BWA (Li and Durbin, 2009). Over 95% of the reads were mapped successfully for all species. However, mapping to the genome of a species that is included in the phylogenetic analysis (in this case the Swan Goose) potentially leads to an inherent bias that could interfere with the phylogenetic analysis. For instance, mean mapping quality (Figure S7.2) is higher for species belonging to the same genus (*Anser*) as the reference genome compared to species of the other genus (*Branta*). Therefore we decided to use the variant calls based on the mapped reads to a more distantly related reference genome, the Mallard (*Anas platyrhynchos*). Although this choice leads to a reduction in the amount of data, it avoids the bias introduced by mapping to the Swan Goose genome, because all species are equally distantly related to the Mallard. A similar approach has been used to reconstruct the phylogeny of Cichlid fish (Ilves and Lopez-Fernandez, 2014).

7.2.3 Phylogenomic Analyses

We applied several phylogenomic methods based on maximum likelihood implemented in RAxML 8.3 (Stamatakis, 2006). We used both supertree and supermatrix techniques (Delsuc et al., 2005). First, the concatenated alignment was analysed under two commonly used substitution models (GTR + Γ and GTR + Γ + I), both with 100 bootstrap replications (Ranwez et al., 2007). Second, we selected 3,570 one-to-one orthologous genes (thus combining the gene exons) with a minimum length of 500 bp. These genes were analysed separately under a GTR + Γ substitution model with 100 rapid bootstraps. The resulting gene trees were filtered on average bootstrap support (minimum > 50). This final set of 3,558 well-supported gene trees was consequently combined into a consensus tree using the software package STELLS version 1.6.1 (Wu, 2012). STELLS infers the species tree from a given set of gene trees, using the multispecies coalescent as underlying genealogical process. To assess statistical support for clades in the supertree analysis, concordance factors were calculated in DensiTree (Bouckaert, 2010). A concordance factor represents the percentage of gene trees that contain the same node as the species tree (Baum, 2007, Knowles and Kubatko, 2010). Trees were rooted either by using an outgroup (Mallard), or by midpoint rooting (without an outgroup). Both methods resulted in the same position of the root in the tree. Here, we present the results of the midpoint rooting only because the high divergence of the root species (Mallard) resulted in conflicting parameter estimations in RAxML 8.3.

7.2.4 Molecular Clock Analyses

Ideally, a molecular clock analysis is run with multiple calibration points (fossils or biogeographic events) using a relaxed clock (Parham et al., 2012). Unfortunately, the fossil record for geese is too sparse to use multiple calibration points (see *7.4 Discussion*). Therefore, we followed the method outlined below. The resulting divergence times should thus not be interpreted as the exact dates of divergence, but rather as rough guiding estimates that allow us to formulate testable hypotheses on the biogeographical and ecological drivers of goose speciation.

We estimated divergence times using an approximate likelihood method as implemented in MCMCtree (in PAML version 4, Yang, 2007), with a global clock and birth-death sampling. We fitted a GTR + Γ model to the concatenated alignment and estimated a mean mutation rate by fitting a strict clock and setting the root age at 9 Mya based on previous studies (Jetz et al., 2012, Fulton et al., 2012). This mean mutation rate (0.134992 ± 0.000307 substitutions/site/Myr) was used to adjust the prior on the mutation rate (rgene) modelled by a Γ distribution as Γ (1, 7.4). Parameters for the birth-death process with species sampling (BDS) and σ^2 values were set at 1 1 0 and G(1,10), respectively. The *Anser-Branta* divergence was constrained between 20 and 4 million years ago to reflect reports of goose fossils in this time period (Brodkorb, 1964). MCMCtree analyses were run multiple times to check for convergence of results. Analyses with a relaxed clock gave similar results compared to the global clock approach, but without convergence of results. Therefore, we only present the estimates of the global clock analysis here. To further assess the reliability of the MCMCtree analysis, we performed a global clock calculation of divergence times using the software package PATHd8 (Britton et al., 2007), setting the root age at 9.5 Mya as a result of the MCMCtree analysis.

7.3. Results

The concatenation and consensus methods resulted in identical topologies (Figure 7.2). All clades in the concatenation analysis were supported by maximum bootstrap values, except for the sisterspecies relation between Tundra Bean Goose and Pink-footed Goose which has a bootstrap support of 95. Analyses with the GTR + Γ substitution model and the GTR + Γ + I substitution model resulted in the same topology. There is a clear separation between the two genera (*Anser* and *Branta*). The genus *Branta* consists of a clade of White-cheeked Geese - Canada Goose (*Branta canadensis*), Cackling Goose (*Branta hutchinsii*), Barnacle Goose (*Branta leucopsis*) and Hawaiian Goose (*Branta sandvicensis*) – and two basal splits – leading to Brent Goose (*Branta bernicla*) and Red-breasted Goose (*Branta ruficollis*). The most basal split in the genus *Anser* leads to the Bar-headed Goose (*Anser indicus*). Next, two main clades can be recognised: the White Geese – Snow Goose (*Anser caerulescens*), Ross' Goose (*Anser rossii*) and Emperor Goose (*Anser canagicus*) – and the Grey Geese – Greylag Goose (*Anser anser*), Swan Goose (*Anser cygnoides*), the White-fronted Geese (*Anser albifrons* and *Anser erythropus*) and the Bean Goose complex (*Anser fabalis, Anser serrirostris* and *Anser brachyrhynchus*). Concordance factors ranged between 4% and 93% (Figure 7.2), with generally higher values found within *Branta* (30% to 93%) than within *Anser* (4% to 54%). Indeed, concordance factors in the consensus analysis were consistently higher for the *Branta* compared to the *Anser* (Mann-Whitney $U = 60.5$, $p = 0.015$), which indicates higher levels of phylogenetic incongruence in the latter clade.

Figure 7.3 gives an overview of the divergence times (with 95% CI) for the different speciation events. The split between *Anser* and *Branta* was estimated at 9.5 Mya (15.1 – 4.2). This estimate was used for root calibration in the PATHd8 analysis, which resulted in similar divergence times compared to the MCMCtree analysis ($R^2 = 0.973$, $p < 0.001$, Figure S7.3). Most mean divergence times are concentrated between 4 and 2 Mya. The mean divergence times for Red-breasted Goose and Brent Goose are dated to approximately 6.8 and 5.8 Mya, respectively. Finally, three Brent Goose subspecies (*bernicla, hrota* and *nigricans*) originated during the Pleistocene, around one Mya. An overview of the evolutionary history of True Geese is presented in Figure 7.4, combining phylogenetic relationships, divergence times and current distributions.

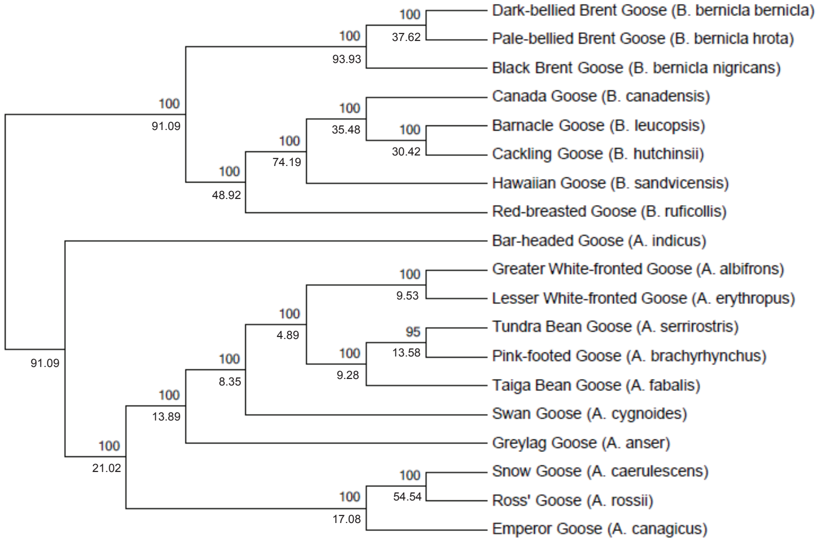


Figure 7.2 Maximum Likelihood Tree for the True Geese based on consensus and concatenation methods. Bootstrap values of the concatenation analysis above branches, concordance factors of the consensus analysis below.

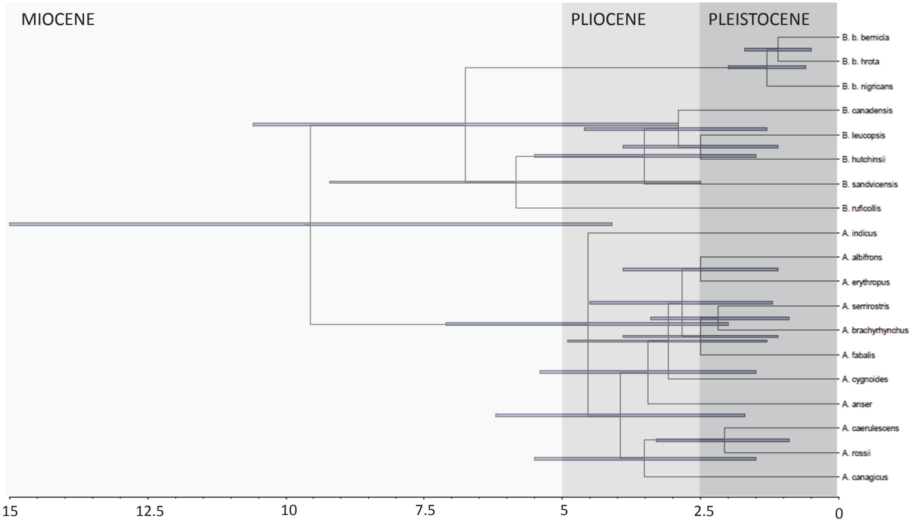


Figure 7.3 Timed phylogenetic tree using a global clock approach in MCMCtree. Blue bars indicate 95% confidence intervals.

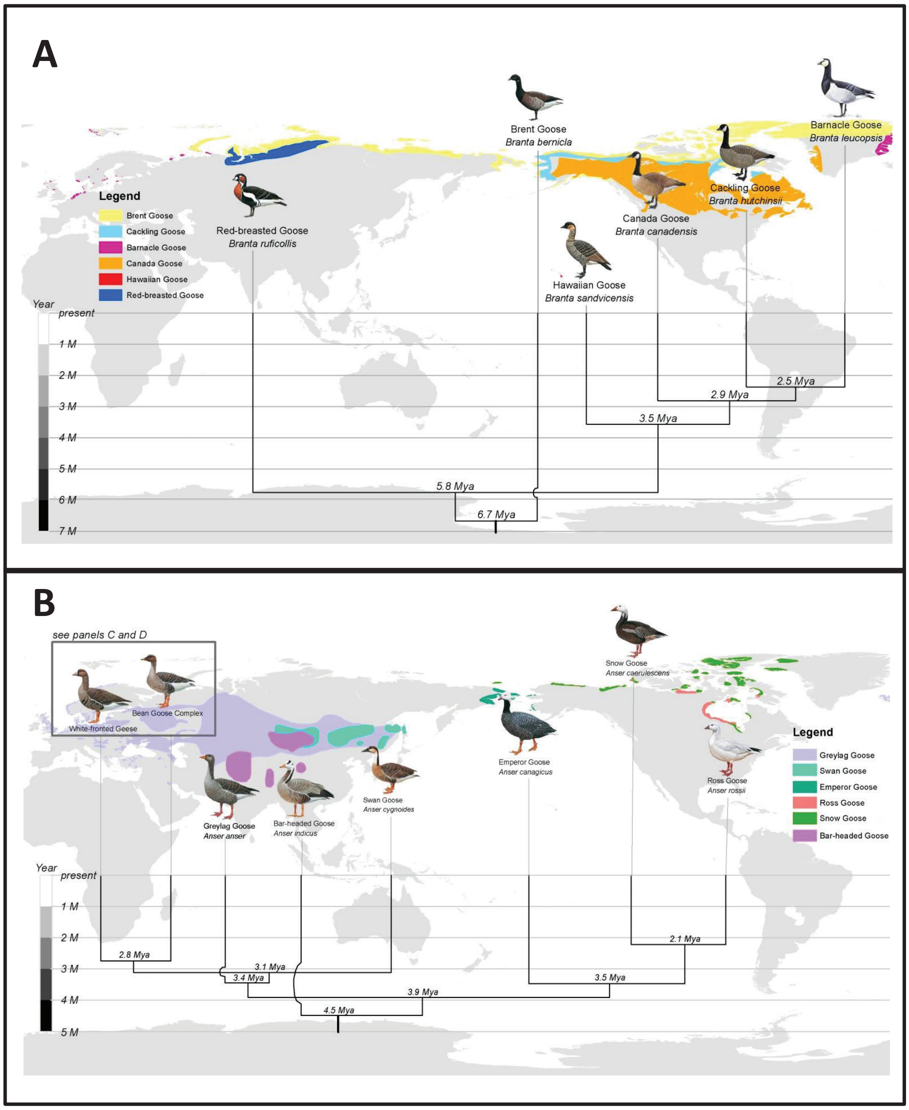


Figure 7.4. An overview of the evolutionary history of the True Geese combining phylogenetic tree, divergence times and current distributions of all species. Distributions based on BirdLife's species range maps. Drawings used with permission of the Handbook of Birds of the World (del Hoyo and Elliott, 1992).

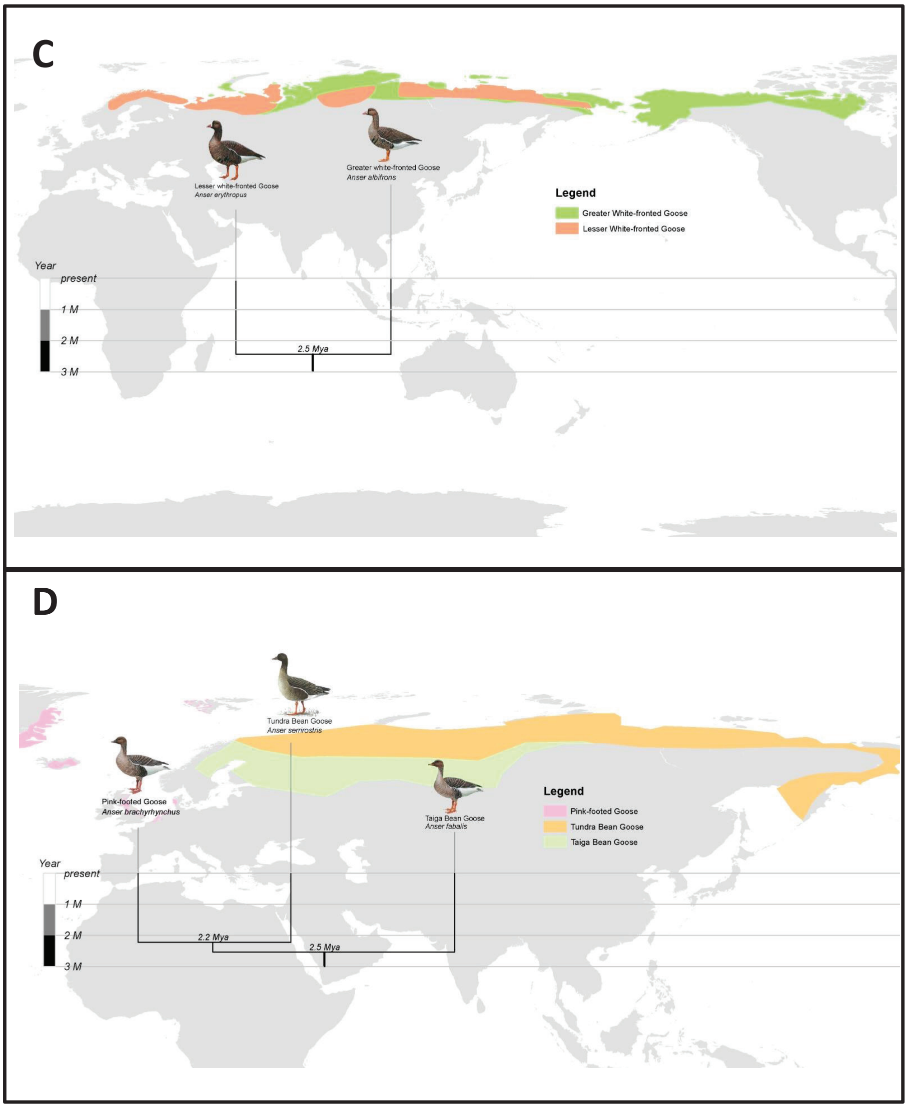


Figure 7.4. ***Continued***

7.4. Discussion

Whole genome analyses have recently been used to unravel the phylogenetic relations between the major bird orders (Jarvis et al., 2014, Prum et al., 2015), but our study presents one of the first phylogenomic analyses of a group of closely related bird species (DaCosta and Sorenson, 2016, Nater et al., 2015). As both concatenation and consensus methods resulted in the same topology and all nodes on the concatenation tree are supported by high bootstrap values, it is likely that the resulting phylogeny closely approaches the actual species tree for the Anserini. We firmly resolve several incongruences among previous studies (see section *7.4.1 Phylogenetic Relationships*).

The resolution of these incongruences can be attributed to the deliberately exon-based phylogenomic approach in our study (Ilves and Lopez-Fernandez, 2014). First, the choice of one-to-one orthologous exonic sequences ensures high mapping quality because of greater sequence conservation of exons compared to introns (Ranwez et al., 2007, Kerr et al., 2014) Moreover, the use of one-to-one orthologous sequences avoids the analytical complexities introduced by duplicated genes (Maddison, 1997) and the occurrence of indels and greater sequence divergence that can pose substantial problems during multiple sequence alignment (Liu et al., 2010). Second, the genome-wide set of thousands of genes enabled us to deduce with high statistical power the species tree despite potentially high levels of phylogenetic incongruence caused by incomplete lineage sorting and/or hybridization. Indeed, both the concatenation and the consensus method uncovered the same phylogenetic signal from the genome-wide sequence data. Furthermore, the concordance factors calculated in the coalescent-based consensus method provide important insights into the effects of incomplete lineage sorting and/or hybridization in certain clades.

7.4.1 Phylogenetic Relationships

Monophyly of the genera *Anser* and *Branta* was already well-established (Delacour and Mayr, 1945). However, the timing of separation of these genera is still a matter of debate. Fossil evidence suggested that these two groups of geese have a common ancestor between 5 and 4 Mya (Brodkorb, 1964, Wetmore, 1956), and this date has been used to calibrate the molecular clock in birds (Shields and Wilson, 1987). The consistency of this molecular clock has been questioned (Weir and Schluter, 2008, van

Tuinen and Hedges, 2001), because subsequent molecular studies reported older dates for the *Anser-Branta* split ranging from 23 to 9 million years ago (Gonzalez et al., 2009, Jiang et al., 2010, Brown et al., 2008, Pereira and Baker, 2006, Jetz et al., 2012, Fulton et al., 2012). We decided to use 9 million years to calibrate the mutation rate, based on recent estimates (Fulton et al., 2012, Jetz et al., 2012). Moreover, we used a fossil constraint between 20 and 4 million years ago to reflect reports of goose fossils in this time period (Brodkorb, 1964). The resulting wide confidence interval for the *Anser-Branta* split in our molecular clock analysis, ranging from 15.1 to 4.2 Mya, indicates that there is still considerable uncertainty for this estimate. This uncertainty is a consequence of the lack of proper fossil calibration points for the Anserini tribe. Although there are numerous goose fossils (Brodkorb, 1964), it is not possible to confidently determine the phylogenetic position of these fossils. A thorough phylogenetic analysis of goose fossils, such as the one recently published for the Charadriiformes (Smith, 2015), is clearly essential in order to achieve better estimates for the divergence times.

Phylogenetic relationships within the genus *Branta* (commonly referred to as the Black Geese) could be unambiguously inferred, consisting of a clade of White-cheeked Geese – Canada Goose, Cackling Goose, Barnacle Goose and Hawaiian Goose – and two basal splits – leading to Brent Goose and Red-breasted Goose. The exact phylogenetic position of the latter two species remained uncertain up to our study: some studies reported a sister species relation (Lee et al., 2008, Paxinos et al., 2002), while others placed either Red-breasted Goose (Livezey, 1996, Donne-Gousse et al., 2002) or Brent Goose at the base of the *Branta*-clade (Gonzalez et al., 2009). We find strong support for a basal split leading to the Brent Goose in *Branta*. Within the White-cheeked Geese-clade there is a clear distinction between Canada Goose and Cackling Goose which were considered large- and small-bodied forms of the same species in the past (Owen, 1980). The significant morphological and molecular divergence between these species reported in previous studies is therefore confirmed by our genomic analysis (Scribner et al., 2003, Van Wagner and Baker, 1990). In addition, we find support for the sister species relation between Cackling Goose and Barnacle Goose described previously (Paxinos et al., 2002).

The Hawaiian Goose was the first species to split from the White-cheeked Geese-clade, around 3.5 Mya (5.5 – 1.5). This date suggests that the ancestors of the Hawaiian Goose arrived on islands that were present in this time period, such as Kauai, Nihoa or Oahu, (Macdonald et al., 1983). However, it is also possible that the divergence of the Hawaiian Goose occurred on the mainland, followed by colonization of Hawaii at a later stage. Our results contrast with the findings of Paxinos et al. (2002), who reconstructed the evolutionary history of the Hawaiian goose radiation (i.e. the modern Hawaiian Goose and several extinct species) using mtDNA extracted from fossil remains. They found that the Hawaiian radiation is a sister group to the Canada Goose that diverged approximately 890,000 years ago (1.3 Mya - 577,000), a date also reported by Quinn et al. (1991) based on restriction fragments of mtDNA. The incongruence between our analysis and those of Paxinos et al. (2002) and Quinn et al. (1991) is probably the result of incomplete lineage sorting. A closer look at the results of the consensus method (which takes into account incomplete lineage sorting) supports this explanation. The clade comprising Canada Goose, Cackling Goose and Barnacle Goose is recovered in 35.5% of the gene trees, whereas a sister relation between Canada Goose and Hawaiian Goose is supported by 16% of the gene trees. Gene tree incongruence can also be the result of hybridization (Maddison, 1997), but this is less likely as the Hawaiian Goose has been geographically isolated from the Canada Goose. However, we cannot rule out the possibility that vagrant Canada Geese might have reached the Hawaiian archipelago in the past (Berger, 1972) or that Canada Geese and Hawaiian Geese have hybridized before the colonization of Hawaii. Another incongruence between our results and those of Paxinos et al. (2002) and Quinn et al. (1991) concerns the timing of divergence of the Hawaiian Goose (3.5 Mya versus 890,000 years ago). This large difference can be explained by the fossil calibration used by these studies as they both calibrated the molecular clock by setting the date of the *Anser-Branta* split at 4.5 Mya, which is later than the date we use here (9.5 Mya). In addition, the effective population size of mtDNA is smaller compared to nuclear DNA, which results in a considerably younger divergence time (Ballard and Whitlock, 2004).Therefore the Hawaiian Goose most likely diverged earlier than expected, before the further diversification of the white-cheeked goose ancestor that gave rise to the Canada Goose, Cackling Goose and Barnacle Goose.

In contrast to *Branta*, the phylogenetic relationships within the genus *Anser* were largely unresolved up to this study. The failure to reconstruct the evolutionary history of this bird group can be attributed to rapid speciation, which results in incomplete lineage sorting, and hybridization (Ruokonen et al., 2000). Indeed, concordance factors in the consensus analysis were lower for the *Anser*-clade compared to the *Branta*-clade, indicating higher levels of gene tree discordance in the former clade. By using genomic data and methods that take into account gene tree discordance, we were able to construct a well-supported phylogeny for the genus *Anser*. The most basal split leads to the morphologically divergent Bar-headed Goose. Next, two main clades can be recognised: the White Geese – Snow Goose, Ross' Goose and Emperor Goose – that are sometimes placed in a separate genus, *Chen* (Gil and Donsker, 2013), and the Grey Geese – Greylag Goose, Swan Goose, the White-fronted Geese and the Bean Goose complex.

The monophyly of the White Geese was already well-established from a morphological perspective (Livezey, 1996), but molecular analyses were unable to recover this clade (Lee et al., 2008, Donne-Gousse et al., 2002, Gonzalez et al., 2009, Paxinos et al., 2002). We were able to confirm the monophyly of this clade, although gene tree discordance was apparent in the consensus method as only 17.1% of the gene trees supported this clade. From a taxonomic point of view the White Geese should be classified in the genus *Anser*, because placing them in the distinct genus *Chen* results in polyphyly of the genus *Anser*.

Within the Grey Geese-clade, the position of the Swan Goose, as a sister species to the White-fronted Geese and the Bean Goose complex, is quite surprising. Given its morphological disparity, the Swan Goose was traditionally placed at the base in the Grey Geese-clade (Livezey, 1996) and has even been classified in a separate genus, *Cygnopis* (Owen, 1980). Also, the phylogenetic position of the Lesser White-fronted Goose has been established as a sister species of the Greater White-fronted Goose. The placement of the Lesser White-fronted Goose in a clade with Taiga Bean Goose and Pink-footed Goose by previous studies can be explained by analytical shortcomings, incomplete lineage sorting and/or hybridization (Lee et al., 2008, Ruokonen et al., 2000).

The taxonomy and evolutionary relationships of the Bean Goose complex have been controversial (Delacour, 1951, Mooij and Zöckler, 1999, Sangster and Oreel, 1996). The current classification encompasses three species: the Pink-footed Goose, the Taiga Bean Goose (three subspecies) and the Tundra Bean Goose (two subspecies). However, based on analyses of the mitochondrial control region, Ruokonen et al. (2008) identified three distinct lineages: the Pink-footed Goose, the Middendorff's Goose (currently a subspecies of Taiga Bean Goose), and the Bean Goose (currently split in Taiga and Tundra Bean Goose). We report a sisterspecies relation between Pink-footed Goose and Tundra Bean Goose. The phylogenetic relationships in this complex are highly influenced by incomplete lineage sorting and/or hybridization. A thorough genomic analysis in a phylogeographic context with broad sampling across the entire range of this species group is needed to confidently reconstruct the evolutionary history of the Bean Goose complex.

7.4.2 Biogeography and Ecology

It can be conceived that True Geese originated in Eurasia, considering the fact that the earliest (Miocene) goose fossils have been excavated in Western Europe (Brodkorb, 1964) and species at the phylogenetically most basal splits (Bar-headed Goose, Brent Goose and Red-breasted Goose) currently have a largely Eurasian distribution (although the current distribution does not necessarily coincide with the past distribution). If we assume an Eurasian origin, at least three colonization events to North America can be recognized: by the White-cheeked Geese, by the White Geese, and finally by the Greater White-fronted Geese. However, this scenario remains to be tested by means of ancestral reconstructions in combination with fossil data. Based on the phylogeny and present-day biogeographic patterns, we can recognize three main groups: the Black Geese (*Branta*) with members in North America and Eurasia, the Grey Geese (*Anser*) with a largely Eurasian distribution, and the North American White Geese (*Anser*).

Within these three groups, there is substantial variation in beak morphology which is most pronounced in the Grey Geese of the genus *Anser*. The Grey Geese can be regarded as an adaptive radiation, because they show "the evolution of ecological and phenotypic diversity within a rapidly multiplying lineage" (Schluter, 2000). To qualify as an adaptive radiation, four criteria should be met: (1) common ancestry, (2) rapid speciation, (3) phenotype-environment correlation, and (4) trait utility (Schluter, 2000). The first two criteria (common ancestry and rapid speciation) are supported by our phylogenomic analyses. All members of the Grey Geese share a common ancestor around 3.4 Mya and diversified into several distinct species over a period of about one million years. A phenotype-environment correlation indicates that differences in phenotypes are associated with the use of different resources or environments. For geese, this correlation has been confirmed by numerous field studies (Fox and Bergersen, 2005, Carriere et al., 1999, Giroux and Bergeron, 1996). For instance, the Taiga Bean Goose has a long thin bill adapted for probing soft substrates of bogs and marshlands to feed on underground plant material, whereas the Pink-footed Goose has a short bill suitable for grazing and seed-stripping (Owen, 1976, Reed, 1976). Finally, the trait utility (i.e. evidence that morphological or physiological traits are indeed useful when used in the associated environment) of beak morphology in geese has been confirmed experimentally (Durant et al., 2003, Heuermann et al., 2011, Zhang et al., 2015). The Grey Geese can thus be regarded as an adaptive radiation in terms of beak morphology.

The diversification in beak morphology is less pronounced in the Black Geese and White Geese, which can be attributed to the fact that some members of the Black Geese (specifically, the White-cheeked Geese) and the White Geese coexisted in North America and consequently affected each other in terms of beak morphology evolution by means of resource competition and consequent character displacement (Schluter, 2000, Dayan and Simberloff, 2005). So, while the Eurasian niches were rapidly filled by members of one ancestral Grey Goose lineage, the North American niches were filled by members of two distantly related lineages: the Black Geese and the White Geese. One can see every North American species as the ecological equivalent of an Eurasian species (or vice versa), for example, Owen (1980) remarked that "the larger races of the Bean Goose [are] the Canada's [goose] ecological counterpart in Eurasia." However, it is also possible that the Black Geese and White Geese both exhibited an adaptive radiation in North America comparable with the Eurasian radiation of the Grey Geese, but that several species went extinct during the Pleistocene (Martin and Klein, 1989).

We hypothesized a Pliocene origin for modern goose species followed by diversification at the subspecies level during the Pleistocene, based on the available fossil evidence (Brodkorb, 1964) and previous molecular studies (Avise et al., 1992, Van Wagner and Baker, 1990, Ruokonen et al., 2000, Scribner et al., 2003). Indeed, our analysis indicates that the majority of speciation events took place at the end of the Pliocene. The approximate date of diversification coincides with the beginning of a period of climatic oscillations between 3.2 and 1.9 million years ago. This period was part of a fast global cooling trend, following the closure of the Panama Seaway and the uplifting of the Tibetan Plateau around 4 million years ago (Zachos et al., 2001). This resulted in the formation of permanent Northern Hemisphere ice sheets, the establishment of a circumpolar tundra belt and the emergence of temperate grasslands, which opened up new ecological niches in which new groups of animals and plants were able to spread (Kahlke, 2014, Prins, 1998). The tundra habitat serves as breeding ground for geese (Owen, 1980), while the temperate grasslands act as wintering grounds where mate choice takes place (Rodway, 2007). Moreover, these tundra and grassland habitats provided ample opportunity for geese to explore new ecological niches and diversify in beak morphology.

7.5. Conclusions

Using a phylogenomic approach we were able to resolve the contentious phylogenetic history of the True Geese. Furthermore, taking advantage of the many contrasting gene histories, we gained more insight into the effects of complex speciation processes, such as rapid diversification and hybridization, in certain clades. The relative importance of hybridization in the evolutionary history of the True Geese remains to be investigated. The widespread occurrence of hybridization in birds (Ottenburghs et al., 2015), and specifically waterfowl (Kraus et al., 2012, Randler, 2008, Ottenburghs et al., 2016c), suggests that hybridization can act as an important component in avian speciation (Rheindt and Edwards, 2011). By integrating over the full exon set of genes we made a first step to quantitatively describe both species and gene histories. Our approach will be a fruitful strategy for resolving many other complex evolutionary histories at the level of genera, species, and subspecies.

7.6. Supplementary Material

All samples were collected from captive birds, except for Greater White-fronted Goose and Barnacle Goose. All breeders ensured us that hybridization is avoided at all times. The wild samples did not show any signs of hybridization. Furthermore, we should have been able to detect recent hybridization events during the analysis, which was not the case.

Table S7.1: Sampled goose species and sampling location

Species		Sampling Location
Common Name	**Scientific Name**	
GENUS ANSER		
Greater White-fronted Goose	*A. albifrons*	Collection Müskens
Lesser White-fronted Goose	*A. erythropus*	Collection Müskens
Greylag Goose	*A. anser*	Collection Brenders
Swan Goose	*A. cygnoides*	Avifauna (Alphen aan de Rijn, NL)
Pink-footed Goose	*A. brachyrhynchus*	NIOO (Wageningen, NL)
Taiga Bean Goose	*A. fabalis*	Collection Müskens
Tundra Bean Goose	*A. serrirostris*	Collection Müskens
Bar-headed Goose	*A. indicus*	Ouwehands Zoo (Rhenen, NL)
Emperor Goose	*A. canagicus*	Collection Meinen
Ross' Goose	*A. rossii*	Avifauna
Snow Goose	*A. caerulescens*	Collection Meinen
GENUS BRANTA		
Dark-bellied Brent Goose	*B. bernicla bernicla*	Collection Meinen
Pale-bellied Brent Goose	*B. b. hrota*	Collection Brenders
Black Brent Goose	*B. b. nigricans*	Collection Meinen
Canada Goose	*B. canadensis*	NIOO (Wageningen, NL)
Cackling Goose	*B. hutchinsii*	Avifauna (Alphen aan de Rijn, NL)
Barnacle Goose	*B. leucopsis*	Collection Müskens
Red-breasted Goose	*B. ruficollis*	Avifauna (Alphen aan de Rijn, NL)
Hawaii Goose	*B. sandvicensis*	Collection Meinen

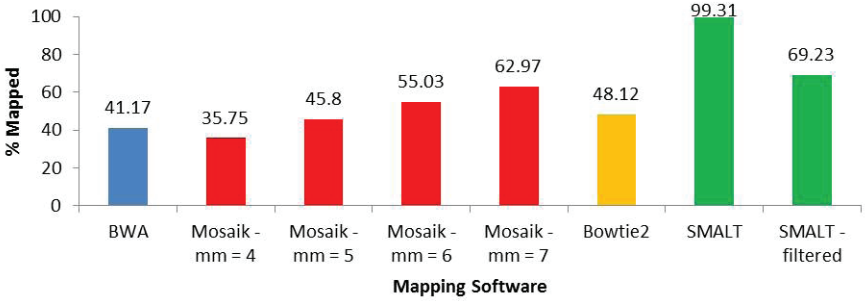


Figure S7.1. Mapping results for different software on the sample of Swan Goose (*Anser cygnoides*).

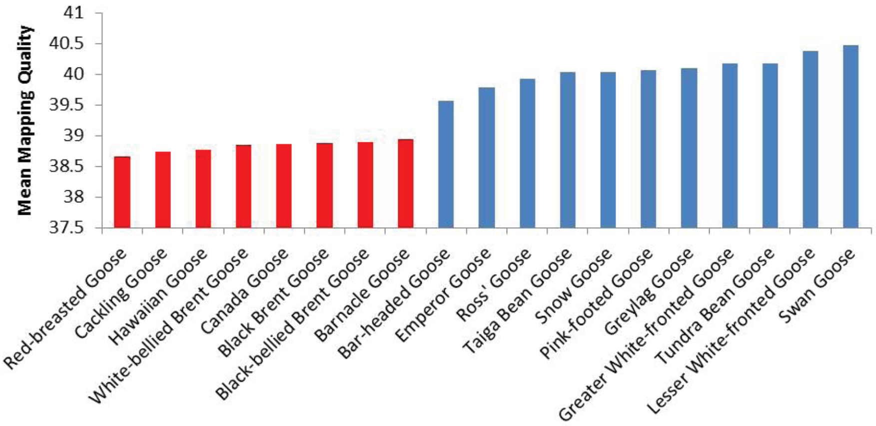


Figure S7.2. Mean Mapping Quality of the paired-end reads to the Swan Goose genome using BWA. Blue (*Anser*) and Red (*Branta*) bars represent the different genera.

Table S7.2. Mapping results of all goose samples to Mallard genome (version 73) using SMALT

Species	N Reads	Reads Mapped	Reads Properly Paired	N reads (after processing*)	Average Depth of Coverage
Swan Goose (*Anser cygnoides*)	248,073,165	246,361,460 (99.31%)	171,741,052 (69.23%)	167,787,462	15.5777
Lesser White-fronted Goose (*Anser erythropus*)	239,825,881	238,147,100 (99.30%)	151,282,166 (63.08%)	147,604,596	13.6891
Greater White-fronted Goose (*Anser albifrons*)	209,540,538	208,157,570 (99.34%)	165,243,668 (78.86)%	168,279,164	15.5885
Pink-footed Goose (*Anser brachyrhynchus*)	243,037,139	240,339,427 (98.89%)	180,819,631 (74.40%)	185,068,118	17.138
Greylag Goose (*Anser anser*)	180,263,790	178,857,732 (99.22%)	134,044,154 (74.36%)	137,523,408	12.8095
Taiga Bean Goose (*Anser fabalis*)	234,490,156	231,934,213 (98.91%)	175,609,678 (74.89%)	182,498,873	16.9499
Bar-headed Goose (*Anser indicus*)	178,281,121	176,034,779 (98.74%)	132,017,170 (74.05%)	134,342,616	12.5142
Tundra Bean Goose (*Anser serrirostris*)	237,129,371	234,141,541 (98.74%)	177,040,788 (74.66%)	181,589,528	16.8361
Snow Goose (*Anser caerulescens*)	151,597,232	150,642,169 (99.37%)	111,151,091 (73.32%)	113,220,052	10.5805
Ross' Goose (*Anser rossii*)	146,841,711	145,681,661 (99.21%)	110,982,965 (75.58%)	115,445,482	10.7958
Emperor Goose (*Anser canagicus*)	152,586,156	151,579,087 (99.34%)	115,370,393 (75.61%)	114,487,545	10.6968
Hawaii Goose (*Branta sandvicensis*)	149,320,696	148,230,655 (99.27%)	113,334,408 (75.90%)	116,924,557	10.9345
Canada Goose (*Branta canadensis*)	149,260,299	148,051,291 (99.19%)	106,661,410 (71.46%)	106,452,024	9.93374
Red-breasted Goose (*Branta ruficollis*)	145,708,845	144,324,611 (99.05%)	99,999,980 (68.63%)	105,189,385	9.84807
Cackling Goose (*Branta hutchinsii*)	146,420,070	145,424,414 (99.32%)	110,883,919 (75.73%)	116,028,146	10.8286
Dark-bellied Brent Goose (*Branta bernicla bernicla*)	149,595,960	148,264,556 (99.11%)	106,437,526 (71.15%)	108,789,885	10.1684
Pale-bellied Brent Goose (*Branta bernicla hrota*)	149,880,011	148,725,935 (99.23%)	107,643,824 (71.82%)	110,483,376	10.3212
Black Brent Goose (*Branta bernicla nigricans*)	149,004,169	147,782,335 (99.18%)	105,867,462 (71.05%)	108,191,843	10.0909
Barnacle Goose (*Branta leucopsis*)	151,975,535	150,942,101 (99.32%)	117,781,040 (77.50%)	118,981,896	11.0964

*** Processing includes removal of duplicate sequences (Samtools) and realignment (GATK)**

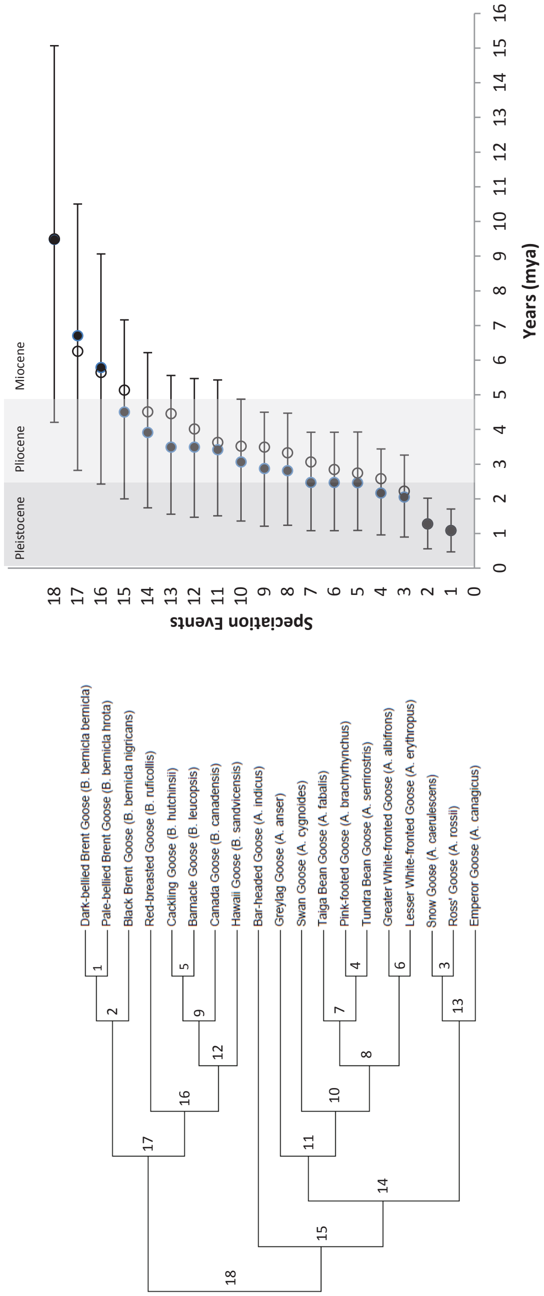


Figure S7.3. Mean divergence times from MCMCtree analysis (black dots) with 95% confidence intervals and from PATHd8 analysis (white dots).

Chapter 8

A history of hybrids: genomic patterns of introgression in the true geese

Ottenburghs, J., Megens, H.-J., Kraus, R.H.S., van Hooft, P., van Wieren, S.E., Crooijmans, R.P.M.A., Ydenberg, R.C., Groenen, M.A.M. & Prins, H.H.T. (2016). A History of Hybrids: Genomic Patterns of Introgression in the True Geese. **Submitted**

Abstract

The impacts of hybridization on the process of speciation are manifold, leading to distinct patterns across the genome. Genetic differentiation accumulates in certain genomic regions, while divergence is hampered in other regions by homogenizing gene flow, resulting in a heterogeneous genomic landscape. A consequence of this heterogeneity is that genomes are mosaics of different gene histories that can be compared to unravel complex speciation and hybridization events. Complex evolutionary histories with rapid speciation (leading to incomplete lineage sorting) and hybridization mostly result in high levels of phylogenetic incongruence (i.e. gene tree discordance), which can be difficult to capture in a traditional, bifurcating phylogenetic tree. Phylogenetic networks can be a powerful tool to display and analyse these evolutionary histories. We unravel hybridization patterns during and after the diversification of the True Geese using a phylogenetic network approach and taking advantage of high levels of phylogenetic incongruence across the whole genome by fully re-sequencing all taxa of this clade. In addition, we determine the timing of putative hybridization events and reconstruct historical effective populations sizes for all goose species to infer which demographic or biogeographic factors can explain the observed patterns of introgression. We find evidence for ancient interspecific gene flow during the diversification of these goose species and we were able to pinpoint several putative hybridization events. The reconstruction of historical effective populations sizes indicate that most species showed a steady increase during the Pliocene and Pleistocene followed by population subdivision during the Last Glacial Maximum about 110,000 to 12,000 years ago. The combination of large effective population sizes and occasional range shifts might have facilitated contact between diverging goose species, resulting in the establishment of numerous hybrid zones and consequent gene flow. Our approach, based on genome-wide phylogenetic incongruence and network analyses, will be a useful procedure to reconstruct the complex evolutionary histories of many naturally hybridizing species groups.

8.1. Introduction

The impacts of hybridization on the process of speciation are manifold (Abbott et al., 2013). Hybridization may slow down or even reverse species divergence. It may also accelerate speciation via adaptive introgression or contribute to species diversity through the formation of new hybrid taxa (Figure 8.1). These diverse effects occur at different spatial scales and during different stages across the speciation continuum. The consequences of hybridization and its role in impeding or promoting speciation are thus expected to vary widely among hybridizing taxa and at different stages of divergence. In every case, the pattern of hybridization is only a single snapshot of a complex and continuously changing interaction.

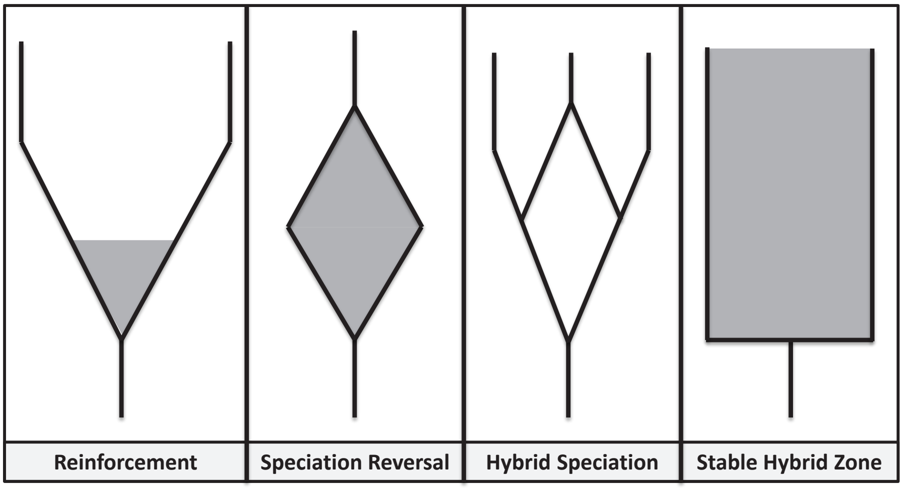


Figure 8.1. Different outcomes of hybridization: reinforcement, speciation reversal, hybrid speciation and the formation of a stable hybrid zone. The grey area indicates introgressive hybridization. Time elapses from bottom to top.

Hybridization between recently diverged taxa can result in reversal of the speciation process: if reproductive isolation between the interacting taxa is incomplete, these taxa might merge into one species again (Seehausen, 2006, Seehausen et al., 2008a). However, hybridization can also contribute to the speciation process by means of reinforcement (Servedio and Noor, 2003, Marshall et al., 2002). Reinforcement is "the evolution of prezygotic isolation mechanisms in zones of overlap or hybridization as a response to selection against hybridization" (Howard, 1993). Although effects and mechanisms of reinforcement are poorly understood, Coyne and Orr (2004) state that "present data and theory show that reinforcement is possible – and must be taken seriously – but they do not show that reinforcement is common, much less ubiquitous."

The outcome of hybridization is mostly studied in the context of hybrid zones, where genetically distinct populations interbreed and produce hybrids (Barton and Hewitt, 1985, Harrison, 1990). Initially, hybrid zones were regarded as transient phenomena, which will ultimately lead to either speciation (through reinforcement, in early literature referred to as "adaptive speciation") or fusion of the two taxa by means of introgressive hybridization (Dobzhansky, 1937, Wilson, 1965). However, hybrid zones can also be stable over long periods of time, for example due to a balance between dispersal of parental taxa into the hybrid zone and selection against hybrids (i.e. tension zones, Barton and Hewitt, 1985). This stable situation can facilitate the exchange of genes between the interacting taxa (Payseur, 2010). The exchanged genes may provide an adaptive advantage for one of the taxa, a phenomenon now termed "adaptive introgression." Botanists have long stressed the importance of introgression in plant evolution (Anderson, 1949) and in recent years adaptive introgression has been documented for several plant taxa (Whitney et al., 2015, Kim et al., 2008, Martin et al., 2006). In animals, several instances of adaptive introgression have been described, including pesticide resistance in mice (Song et al., 2011), coat colour in wolves (Anderson et al., 2009), and mimicry patterns in *Heliconius* butterflies (Dasmahapatra et al., 2012).

The adaptive role of hybridization can be especially apparent in adaptive radiations (Seehausen, 2004). During an adaptive radiation many closely related taxa may interact, increasing the chances for hybridization and consequent introgression. High levels of genetic exchange increase standing genetic variation, enabling rapid adaptive evolution (Hedrick, 2013). Furthermore, hybrids often exhibit novel or extreme characters compared to their parental taxa, a phenomenon referred to as transgressive segregation (Rieseberg et al., 1999, Dittrich-Reed and Fitzpatrick, 2013). In later stages of the radiation occasional hybridization could facilitate further ecological diversification, which has been suggested for the rapid evolution of mimicry patterns in *Heliconius* butterflies (Gilbert, 2003). On the one hand, hybridization can cause adaptive radiations by transgressive segregation and increasing genetic variation (Seehausen, 2004), while, on the other hand, hybridization might be a consequence of rapid radiations if diversification was faster compared to the evolution of pre- and postzygotic isolation mechanisms (Wiens et al., 2006). Seehausen (2004) called upon the "syngameon hypothesis" when referring to the interplay between hybridization and adaptive radiation. The concept of a syngameon, introduced by Lotsy (1925), has been used extensively in the botanic literature. For example, Grant (1981) described a syngameon as follows: "The definition of the biological species as the most inclusive breeding group does not hold up in cases of naturally hybridizing species and semispecies. Where limited gene exchange is taking place between otherwise isolated semispecies, the most inclusive unit of interbreeding is not a single biological species but an assemblage of semispecies. Such an assemblage is called a syngameon."

Given widespread occurrence of hybridization among birds (Ottenburghs et al., 2015), the syngameon provides a useful framework to study and describe avian evolution in many clades. Avian examples of such groups include Darwin's Finches (Lamichhaney et al., 2015), *Larus* Gulls (Sonsthagen et al., 2012), *Anas* Ducks (Kraus et al., 2012) and North American Wood Warblers of the Parulidae family (Willis et al., 2014). These groups present the opportunity to study the various effects of hybridization (e.g., speciation reversal, reinforcement, adaptive introgression) and their interactions during the evolutionary history of the involved taxa, leading to the species diversity we see today.

Genomics has become a standard practise, also in ornithology (Toews et al., 2016, Kraus and Wink, 2015), opening avenues to answer longstanding questions in speciation and hybridization (Jarvis, 2016, Seehausen et al., 2014). Studies in speciation and hybridization genomics revealed that levels of genetic differentiation between species can be highly variable across the genome: genetic differentiation accumulates in certain genomic regions, while divergence is hampered in other regions by homogenizing gene flow, resulting in a heterogeneous genomic landscape (Nosil et al., 2009, Nosil and Feder, 2012b, Harrison and Larson, 2016). A consequence of this heterogeneity is that genomes are mosaics of different gene histories (Ellegren et al., 2012, Maddison, 1997, Payseur, 2010) that can be compared to unravel complex speciation and hybridization events (Ottenburghs et al., 2016a, Harrison and Larson, 2014).

Complex evolutionary histories with rapid speciation (leading to incomplete lineage sorting) and hybridization mostly result in high levels of phylogenetic incongruence (i.e. gene tree discordance), which can be difficult to capture in a traditional, bifurcating phylogenetic tree. Phylogenetic networks can be a powerful tool to display and analyse these evolutionary histories (Ottenburghs et al., 2016b, Huson and Bryant, 2006). For example, Suh et al. (2015) quantified the amount of incomplete lineage sorting along the Neoaves phylogeny (Jarvis et al., 2014) using presence/absence data for 2,118 retrotransposons and concluded that the "complex demographic history [of the Neoaves] is more accurately represented as local networks within a species tree."

Here, we will unravel hybridization patterns during and after the diversification of the True Geese, a group of naturally hybridizing bird species (McCarthy, 2006, Ottenburghs et al., 2016c). The True Geese are classified in the waterfowl tribe Anserini and have been traditionally divided over two genera: *Anser* and *Branta* (Delacour and Mayr, 1945). Hybrids have been reported within each genus (Delnicki, 1974, Hatch and Shortt, 1976, Leafloor et al., 2013, Nijman et al., 2010, Trauger et al., 1971, Weckstein et al., 2002, Lehmhus and Gustavsson, 2014), but also intergeneric hybrids have been documented (Craven and Westemeier, 1979, Nelson, 1952, Prevett and Macinnes, 1973, Gustavsson, 2009). Previous studies suggested that the evolutionary history of the True Geese is heavily influenced by hybridization and rapid diversification (Ruokonen et al., 2000, Ottenburghs et al., 2016a). The *Anser*-clade can be regarded as an adaptive radiation and was probably affected more by hybridization compared to the more gradually diversifying *Branta*-clade (Ottenburghs et al., 2016a). In this paper, we focus on the role of hybridization during the diversification of these genera using a network approach and taking advantage of phylogenetic incongruence across the whole genome by fully re-sequencing all taxa of the True Geese clade. Specifically, we set out the answer the following questions: (1) Has there been gene flow between different goose species? (2) If so, is this ancient or recent gene flow? And (3) can these patterns of gene flow be explained by demographic, behavioural or biogeographical factors?

8.2. Material and Methods

8.2.1 Genomic Dataset

The workflow that resulted in the present genomic dataset is described in Chapter 7. This dataset is comprised of 41,736 unique exons, representing 5,887 genes. The total alignment (6,630,626 base pairs) was used in the neighbour-joining network and D-statistic analyses described below. In addition, we selected 3,570 one-to-one orthologous genes with a minimum length of 500 bp. These genes were analysed separately under a GTR + Γ substitution model with 100 rapid bootstraps in RAxML version 8.3 (Stamatakis, 2006). The resulting gene trees were filtered on average bootstrap support (minimum > 50). This final set of 3,558 well-supported gene trees was used in the analysis to construct hybridization networks and determine the timing of gene flow.

8.2.2 Gene Flow Analysis

The D-statistic is a statistical test that was first employed to quantify the amount of genetic exchange between Neanderthals and humans (Green et al., 2010). It exploits the asymmetry in frequencies of two nonconcordant gene trees in a three-population setting (Durand et al., 2011). Consider three populations (P_1, P_2 and P_3) and an outgroup (O), of which P_1 and P_2 are sister clades. In this ordered set op populations [P_1, P_2, P_3, O], two allelic patterns are of interest: "ABBA" and "BABA". The pattern ABBA refers to the situation in which P_1 has the outgroup allele "A" and P_2 and P_3 share the derived allele "B", while the pattern BABA refers to the situation in which P_2 has the outgroup allele "A" and P_1 and P_3 share the derived allele "B". Under the null hypothesis that P_1 and P_2 are more closely related to each other than to P_3, and if the ancestral populations of P_1, P_2, P_3 were panmictic, then it is expected that the derived alleles in P_3 match the derived alleles in P_1 and P_2 equally often (Tajima, 1983, Hudson, 1983). In other words, the patterns ABBA and BABA should occur in equal frequencies and the D-statistic should equal zero.

Gene flow between P_1 and P_3 (indicated by an overrepresentation of BABA) or P_2 and P_3 (indicated by an overrepresentation of ABBA) result in a D-statistic that is significantly different from zero. For both genera, D-statistics were calculated for all possible combinations of three species in the program HybridCheck version 1.0.1 (Ward and van Oosterhout, 2016). We combined all species of the other genus as the outgroup. To test for significance, we performed jackknife resampling using blocks of 50,000 bp.

To infer the timing of gene flow (during or after the diversification), we dated 3,558 gene trees using the software PATHd8 version 1.0 (Britton et al., 2007), setting the divergence time between the genera at 9.5 million years ago (based on previous estimates, Fulton et al., 2012, Jetz et al., 2012). For every species pair, histograms were constructed from the resulting divergence times (Li et al., 2016a). The patterns expected when gene flow occurred during or after the diversification are presented in Figure 8.2.

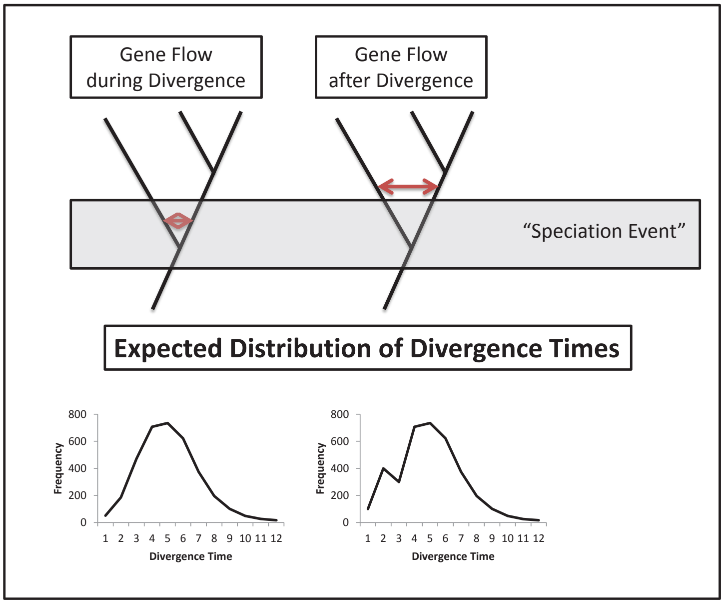


Figure 8.2. Expected distribution of divergence times. If gene flow occurred during the diversification process, it will be indistinguishable from genetic divergence at other loci, resulting in a single peak (left graph). If, on the other hand, gene flow occurred after the diversification process, introgressed loci will show more recent divergence times, which becomes apparent as a recent, smaller peaks (right graph).

8.2.3 Network Analyses

The phylogenomic analysis of the True Geese in Chapter 7 indicated high levels of gene tree discordance, which can be caused by hybridization and/or incomplete lineage sorting. To visualize this phylogenetic incongruence, we constructed a phylogenetic neighbour-joining network using the ordinary least squares method (with default settings) in SplitsTree version 4.1.4.2 (Huson and Bryant, 2006). This network was based on genetic distances, which were calculated in RAxML 8.3 with a GTR + Γ substitution model (Stamatakis, 2006, Ottenburghs et al., 2016a). We calculated the degree distributions (i.e. the number of connections for each node in a network) for each genus to quantify the complexity of the networks using the R-package igraph (Csardi and Nepusz, 2006). The degree distributions for each genus were compared by means of a general linear model with Poisson distribution in R version 3.2.2.

Hybridization networks are networks that attempt to reconstruct a phylogenetic tree with the fewest amount of hybridization events (Chen et al., 2012, Huson and Bryant, 2006). For each genus, we combined 3,558 gene trees into hybridization networks using the Autumn algorithm (Huson and Linz, 2016) with default settings in Dendroscope version 3.4.4 (Huson and Scornavacca, 2012).

8.2.4 Demographic Analysis

We conducted a demographic analysis using a hidden Markov model approach as implemented in the software package PSMC (Li and Durbin, 2011). A consensus sequence was generated from BAM files using the 'pileup' command in SAMtools. For the PSMC analyses, we used the parameter settings suggested by Nadachowska-Brzyska et al. (2015), namely "N30 –t5 –r5 –p 4+30*2+4+6+10."

8.3. Results

8.3.1 Gene Flow Analysis

The D-statistic analysis supported gene flow between several goose species. If multiple tests (with different combinations of three species) indicated gene flow between two particular species, we only report the highest D-statistic in Figures 8.4 and 8.5 (for details see Table S8.1). In the *Branta*-clade, the Red-breasted Goose exchanged genetic material with four other species (Canada Goose, Cackling Goose, Brent Goose and Hawaiian Goose). In addition, there is evidence for gene flow between Cackling Goose and Canada Goose, and between Cackling Goose and Brent Goose. Significant D-statistics in the genus *Branta* varied from 0.0725 to 0.146 (Figure 8.3).

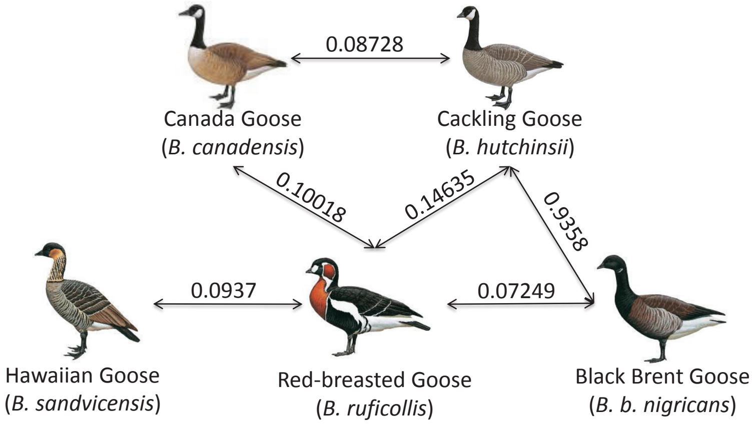


Figure 8.3. Introgression network for the genus *Branta*, based on significant D-statistics (above arrows). Drawings used with permission of Handbook of Birds of the World (del Hoyo and Elliott, 1992).

In the *Anser*-clade, Lesser White-fronted Goose hybridized with five other species (Swan Goose, Snow Goose, Ross' Goose, Greylag Goose and Emperor Goose), Greater White-fronted exchanged genetic material with Emperor Goose and Pink-footed Goose, and the Greylag Goose interbred with Snow Goose and Ross' Goose (Figure 8.4). Significant D-statistics in the *Anser* genus varied from 0.0703 to 0.174. Although the D-statistics for *Anser* were slightly higher compared to *Branta*, there was no significant difference (Mann Whitney U, W = 4659, $p = 0.08826$).

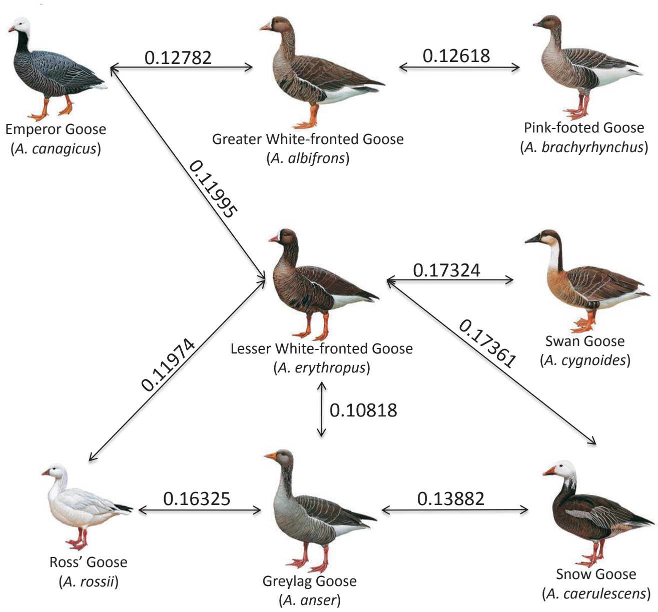


Figure 8.4. Introgression network for the genus *Anser*, based on significant D-statistics (above arrows). Drawings used with permission of Handbook of Birds of the World (del Hoyo and Elliott, 1992).

To infer the timing of gene flow (during or after the diversification), we took advantage of gene tree discordance and constructed histograms based on divergence times of 3,558 gene trees. All analyses supported a scenario of gene flow during divergence with low levels of recent gene flow, because the histograms based on gene tree divergence times all displayed one peak corresponding to the initial species split. The divergence time of several gene trees was close to zero, suggesting low levels of recent gene flow between certain species. Figure 8.5 shows two examples, involving the Cackling Goose and the Lesser White-fronted Goose (for other species, see Figure S8.1).

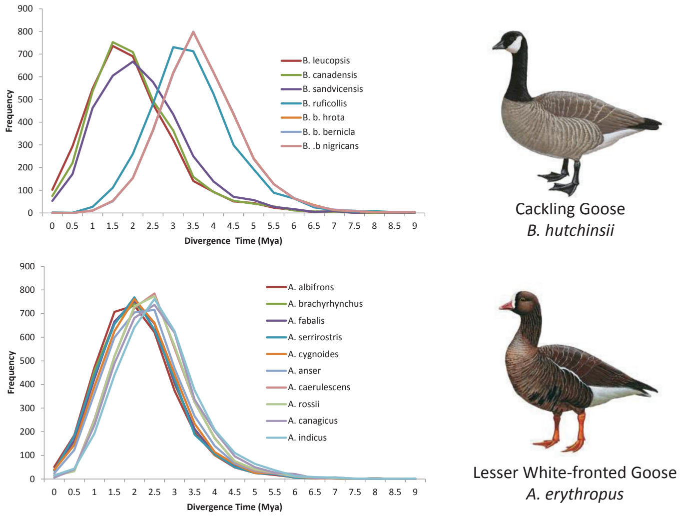


Figure 8.5. Distribution of divergence times for Lesser White-fronted Goose with all *Anser* species and for Cackling Goose with all *Branta* species. All distributions show a single peak, indicating gene flow during divergence. The divergence time of several gene trees was close to zero, suggesting low levels of recent gene flow between certain species.

8.3.2 Network Analyses

The phylogenetic neighbour-joining network (Figure 8.6) based on genetic distances uncovered two main clades that corresponded to the genera *Anser* and *Branta*. Within these clades, the relationships correspond to the phylogenetic analyses of Chapter 7. The comparison of degree distributions revealed that the *Anser*-network was more complex compared to the *Branta*-network (Poisson regression, SD = 0.1908, *z-value* = -5.08, *p-value* < 0.001), because the *Anser*-network contains more nodes with 4 or 5 edges compared to the *Branta*-network. The complexity of the networks was consistent with the suggestion that the evolutionary history of the *Anser*-clade is more heavily influenced by rapid diversification and hybridization compared to the *Branta*-clade.

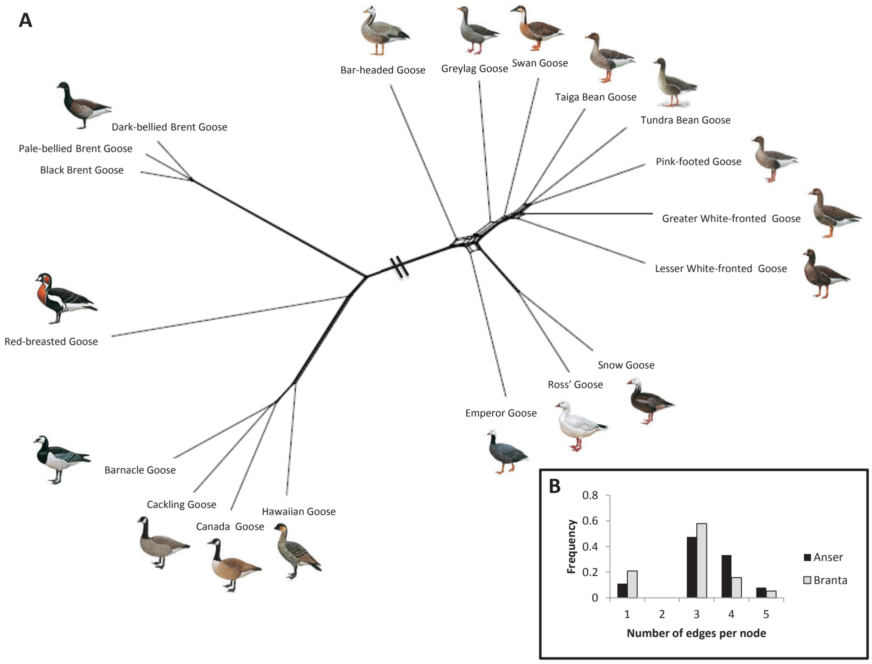


Figure 8.6. (A) Neighbour-joining Network of the True Geese using the ordinary least squares method (with default settings) in SplitsTree version 4.1.4.2 (Huson and Bryant, 2006). based on genetic distances. (B) The comparison of degree distributions indicates that the *Anser*-network is more complex compared to the *Branta*-network as it contains relatively more nodes with 4 and 5 edges. Drawings used with permission of Handbook of Birds of the World (del Hoyo and Elliott, 1992).

We combined 3,558 gene trees into hybridization networks for both genera. Hybridization network analyses of the genus *Anser* did not result in most likely scenarios, underlining the complexity of introgression patterns among *Anser* species. In the genus *Branta*, the hybridization network analyses recovered three (not mutually exclusive) scenarios, indicating hybridization events between the Red-breasted Goose and the ancestor of the White-cheeked Geese (i.e. Hawaiian Goose, Canada Goose, Cackling Goose and Barnacle Goose) and between Red-breasted Goose and Brent Goose (Figure 8.7A-B). In addition, one hybridization network (Figure 8.7C) suggested a hybrid origin for the Red-breasted Goose.

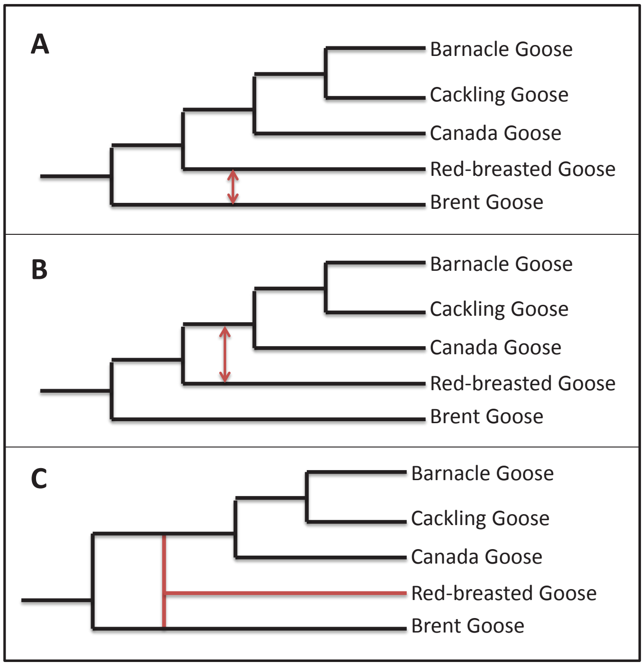


Figure 8.7. Hybridization networks for the genus *Branta* based on the Autumn algorithm (Huson and Linz, 2016) in Dendroscope version 3.4.4 (Huson and Scornavacca, 2012). Network A suggests hybridization between Red-breasted Goose and Brent Goose, network B between Red-breasted Goose and the ancestor of the White-cheeked Geese. Network C suggests a hybrid origin for the Red-breasted Goose.

8.3.3 Demographic analysis

We reconstructed historical effective populations sizes (N_e) for all goose species using the pairwise sequentially Markovian coalescent (PSMC) approach over a range from 1-10 million years ago until about 10,000 years ago. Most *Anser* species (Greater White-fronted Goose, Lesser White-fronted Goose, Tundra Bean Goose, Taiga Bean Goose, Pink-footed Goose, Swan Goose, Greylag Goose, Bar-headed Goose, Snow Goose, and Ross' Goose) and several *Branta* species (Canada Goose, Cackling Goose, Red-breasted Goose, Pale-bellied Brent Goose and Black Brent Goose) show a steady population increase followed by a dramatic expansion, which suggests population subdivision and occasional gene flow, leading to higher levels of heterozygosity and consequently higher estimates of N_e (Jeong et al., 2014, Li and Durbin, 2011). Four species

(Hawaiian Goose, Emperor Goose, Barnacle Goose and Dark-bellied Brent Goose) show clear signs of a bottleneck. Figure 8.8 shows these two patterns as illustrated by Greater White-fronted Goose and Hawaiian Goose (for other species, see Figure S8.2).

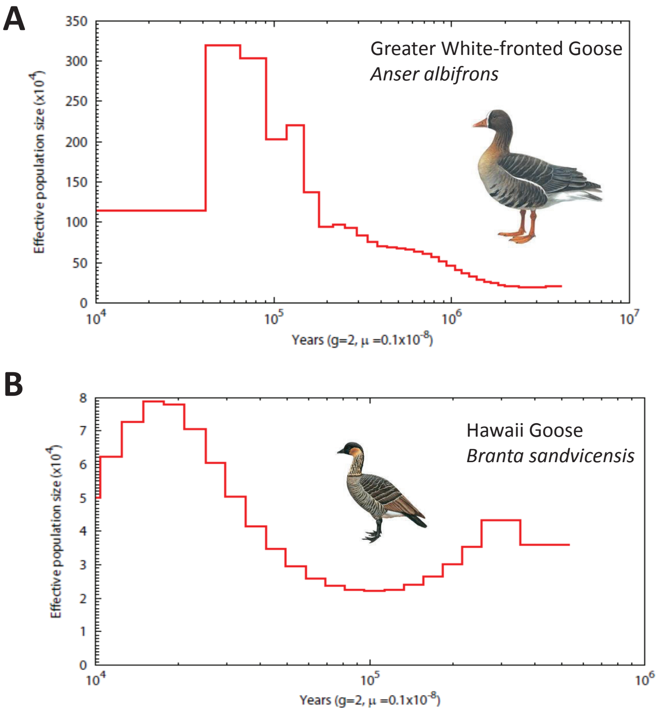


Figure 8.8. Examples of two general demographic patterns for the True Geese based on PSMC analyses. (A) Steady population increase followed by dramatic expansion which suggests population subdivision, as illustrated by Greater White-fronted Goose. (B) Population bottleneck after island colonization, as illustrated by Hawaiian Goose.

8.4. Discussion

8.4.1 General Patterns of Introgression

Based on D-statistics, calculated from genome-wide data, we found evidence for high levels of interspecific gene flow between several goose species. The significant D-statistics varied from 0.07 to 0.17, which is slightly higher compared to analyses on recent radiations, such as Darwin's Finches (0.004 - 0.092; Lamichhaney et al., 2015) and butterflies of the genera *Heliconius* (0.04; Dasmahapatra et al., 2012) and *Papilio* (0.04; Zhang et al., 2013). These values do fall within the range of studies on other hybridizing species, such as pigs (0.11 - 0.23; Frantz et al., 2013), bears (0.04 - 0.46; Liu et al., 2014, Cahill et al., 2015) and *Xiphophorus* fish (0.03 - 0.56; Cui et al., 2013). A significant D-statistic does not necessarily indicate introgression between the species from which the genomes are being compared. There might have been gene flow with an extinct (not sampled) population or the signal might be a remnant from an older hybridization event (Durand et al., 2011, Schumer et al., 2016). For example, the analyses in the *Branta*-clade suggested hybridization between Red-breasted Goose and three other species (Hawaiian Goose, Canada Goose and Cackling Goose). The hybridization network analysis, however, revealed that these significant D-statistics were caused by the signal from an ancient hybridization event between Red-breasted Goose and the ancestor of these three species. Many of the significant D-statistics in the *Anser*-clade can probably be explained in the same way, but the complexity of introgression patterns in this clade did not allow us to pinpoint putative hybridization events.

When did this gene flow occur? Further analyses, based on the divergence times of 3,558 gene trees, indicated that this gene flow was largely due to ancient hybridization during the diversification of these species. Ancient gene flow has been reported for a variety of taxa (Li et al., 2016a, Kutschera et al., 2014, Brennan et al., 2016), including several bird groups (Fuchs et al., 2013, McCormack and Venkatraman, 2013, Rheindt et al., 2009, Wang et al., 2014, Lamichhaney et al., 2015). For instance, Fuchs et al. (2013) attributed a conflicting pattern between several loci to ancient hybridization between members of the woodpecker genus *Campephilus* and the melanerpine lineage (*Melanerpes* and *Sphyrapicus*). The increasing number of studies reporting ancient gene flow during species diversification (Pinho and Hey, 2010) shows that the speciation process is often more complex than, for example, the classical allopatric speciation model (Dobzhansky, 1937, Mayr, 1942).

In the allopatric speciation model, populations become geographically isolated and diverge by genetic drift and/or differential selection pressures, resulting in intrinsic reproductive isolation due to the accumulation of Dobzhansky-Muller incompatibilities (Muirhead and Presgraves, 2016, Coyne and Orr, 2004). This speciation model predicts that the distribution of interspecific divergence is largely determined by a single, shared species split (Wilkinson-Herbots, 2008). But speciation is often more complex: in some cases, speciation may advance by divergent ecological or sexual selection in the face of ongoing gene flow (Nosil, 2012), while, in other cases, allopatrically diverging populations may come into secondary contact and hybridize before reproductive isolation is complete (Seehausen et al., 2014). These more complex speciation models predict that interspecific divergence varies considerably across the genome (Nosil and Feder, 2012b, Nosil et al., 2009), because some genomic regions reflect the initial species split time, whereas others indicate more recent genetic exchange (Wu, 2001, Harrison and Larson, 2014, Payseur, 2010).

With regard to the evolutionary history of geese, we found support for a complex speciation model with high levels of gene flow during species diversification. It is, however, not possible to determine whether this gene flow is the outcome of (repeated) secondary contact or divergence-with-gene-flow. ABC modelling based on multiple samples per species allows for the comparison of several scenarios that differ in the amount and timing of gene flow and can thus be used to confidently discriminate between divergence-with-gene-flow and secondary contact (Smyth et al., 2015, Raposo do Amaral et al., 2013, Yeung et al., 2011). For example, Nadachowska-Brzyska et al. (2013) compared 15 models (with different patterns and levels of gene flow) to assess the demographic history of Pied Flycatcher (*Ficedula hypoleuca*) and Collared Flycatcher (*Ficedula albicollis*). Whole genome re-sequencing data from 20 individuals supported a recent divergence with unidirectional gene flow from Pied Flycatcher into Collared Flycatcher after the Last Glacial Maximum, indicating that the hybrid zone between these species is a secondary contact zone.

Next to evidence for ancient gene flow, we found low levels of recent gene flow, which can be explained in two ways. First, the D-statistic analysis may be unable to detect recent gene flow. Indeed, the D-statistic was developed to detect ancient gene flow and to estimate the extent of archaic ancestry in the genomes of extant populations (Durand et al., 2011). The detection and quantification of recent gene flow warrants a population genomic approach whereby multiple individuals of one population are sequenced (Lavretsky et al., 2016, Toews et al., 2016, Ellegren et al., 2012, Poelstra et al., 2014). Second, the relative rarity of goose hybrids diminishes the opportunity for backcrossing and introgression, leading to absence or low levels of recent gene flow (Ottenburghs et al., 2016c). Third, there may be little recent gene flow because of strong intrinsic and/or extrinsic selection against goose hybrids. Although most goose hybrids are viable and fertile (Ottenburghs et al., 2016c), second generation hybrids or backcrosses may be impaired by genetic incompatibilities (Arrieta et al., 2013, Lijtmaer et al., 2003), or hybrids might be ecologically maladapted (e.g., intermediate beak morphology) or unable to find a mate (Coyne and Orr, 2004). To answer these questions, field observations are needed, which is challenging given the relative rarity of hybrids (Ottenburghs et al., 2016c) and the difficulty of identifying certain hybrids (Randler, 2004). Strong selection against hybrids might also suggest that the diversification of the True Geese was partly driven by reinforcement (Servedio and Noor, 2003).

8.4.2 Demographic Patterns

The reconstruction of historical effective populations sizes (N_e) for all goose species using the pairwise sequentially Markovian coalescent (PSMC) approach indicated two main patterns. First, most species showed a steady increase during the Pliocene and Pleistocene followed by population subdivision (apparent as a dramatic increase in N_e) during the Last Glacial Maximum (LGM, about 110,000 to 12,000 years ago). The increase in population size during the Pliocene and Pleistocene can be explained by a global cooling trend which resulted in the formation of a circumpolar tundra and the emergence of temperate grasslands (Zachos et al., 2001, Prins, 1998, Kahlke, 2014). The tundra habitat acted as breeding ground (Owen, 1980), whereas the grasslands served as wintering grounds where mate choice occurred (Rodway, 2007), enabling goose populations to proliferate. In addition, the climatic fluctuations during the Pliocene and Pleistocene might have instigated range expansions and shifts. This combination of large N_e and occasional range shifts might have facilitated contact between the diverging goose species, resulting in the establishment of numerous hybrid zones and consequent gene flow (Chunco, 2014, Buggs, 2007).

During the LGM many plant and animal populations were subdivided into separate refugia by the ice sheets that expanded from the north (Hewitt, 2000, Hewitt, 1996). This population subdivision has been described for several goose species

(Ploeger, 1968) and the genetic signature of this subdivision has been uncovered for certain species, such as Pink-footed Goose (Ruokonen et al., 2005), Bean Goose (Ruokonen et al., 2008), Greater White-fronted Goose (Eda et al., 2013, Ely et al., 2005), Canada Goose (Scribner et al., 2003), and Snow Goose (Quinn, 1992, Weckstein et al., 2002).

Four goose species colonized islands habitats: the Hawaiian Goose reached the Hawaiian archipelago (Paxinos et al., 2002), the Emperor Goose settled on the Aleutian Islands (Eisenhauer and Kirkpatrick, 1977), and the Barnacle Goose and the Dark-bellied Brent Goose established populations on arctic islands in the North Atlantic, such as Svalbard and Novaya Zemlya (Madsen et al., 1999). In all four cases, the decrease in N_e and consequent genetic bottleneck caused by this island colonization are apparent in the PSMC analyses. It is well-established that island colonization leads to a reduction in heterozygosity and N_e (Nei et al., 1975), and that islands populations have lower levels of genetic variation compared to mainland species (Frankham, 1997). Genetic bottlenecks following islands colonization have been documented for numerous other bird species (e.g., Spurgin et al., 2014a, Clegg et al., 2002).

8.4.3 Comparing Anser and Branta

There is a striking contrast in the patterns of introgression between the two genera. The general network analysis showed that the *Anser*-network is more complex than the *Branta*-network. And D-statistics were slightly (although not significantly) higher in the *Anser*-clade. While high levels of gene flow hindered the precise reconstruction of hybridization events in the *Anser*-clade, it was possible to pinpoint several putative hybridization events within *Branta*-clade. The hybridization network analyses provided evidence for gene flow between the Red-breasted Goose and the ancestor of the White-cheeked Geese (i.e. Hawaiian Goose, Canada Goose, Cackling Goose and Barnacle Goose), between Red-breasted Goose and Brent Goose, and between Canada Goose and Cackling Goose. Past gene flow between the latter two species has been reported previously (Leafloor et al., 2013). What factors can explain the differential introgression patterns between *Anser* and *Branta*? We will consider three possible factors: (1) macro-evolutionary dynamics, (2) morphological and behavioural differences, and (3) demographic dynamics.

First, these patterns of introgression were reconstructed by comparing the genomes of modern, extant species. The ancestors of these modern species may have interbred with unknown extinct species. It might thus be possible that the evolutionary history of the *Branta*-clade was influenced by hybridization as much as the diversification of the *Anser*-clade, but that many *Branta*-species have become extinct. For example, the Hawaiian radiation of *Branta* geese consisted of at least three species, of which only the Hawaiian Goose remains today (Paxinos et al., 2002). The different introgression patterns (as observed by comparing extant genomes) could then be attributed to differences in extinction rates between the genera. Unfortunately, the fossil record for geese is currently still too sparse to test this hypothesis (Mlíkovský, 2002, Brodkorb, 1964).

Second, differential introgression patterns may be explained by differences in behaviour (Wirtz, 1999, Randler, 2006). Although the behaviour of extant species does not necessarily correspond to the ancestral behaviour, we can speculate about possible differences between the genera. Pair formation, involving several pre-copulatory displays, and copulation vary little between the species and the genera (Owen, 1980, Johnsgard, 1965), which can explain the frequent occurrence of hybridization on the species level, but does not clarify the differences in introgression patterns between the genera. Are there differences in certain behaviours that lead to hybridization, such as interspecific nest parasitism or forced extra-pair copulations (Randler, 2005)? These behaviours have been observed in both genera, but the relative contribution of each behaviour to the occurrence of goose hybrids remains to be quantified (Ottenburghs et al., 2016c).

Mate choice in waterfowl is largely determined by sexual imprinting (Rohwer and Anderson, 1988). *Anser* species are morphologically more similar compared to *Branta* species, which might increase the probability of heterospecific mate choice. Based on this reasoning, we expect more *Anser* hybrids compared to *Branta*. This expectation remains to be tested, but will be challenging because hybrids between morphologically similar species are difficult to identify (Randler, 2004) and many goose hybrids are probably of captive origin (Ottenburghs et al., 2016c).

Third, differences in demographic dynamics, mediated by a particular biogeographical and climatic context, might determine the frequency of interspecific interactions, possibly leading to introgressive hybridization. The *Anser*-clade has a largely Eurasian distribution (with the exception of Snow Goose and Ross' Goose).

The open tundra landscape of Eurasia during the Pleistocene allows for large effective population sizes and the climatic fluctuations during the Pliocene and Pleistocene might have instigated range expansions and shifts. In contrast to the *Anser*-clade, the *Branta* species are more widely distributed across the Northern Hemisphere: Canada Goose and Cackling Goose in North America, Hawaiian Goose on the Hawaiian islands, Barnacle Goose and Red-breasted Goose in Eurasia, and the circumpolar Brent Goose. This distribution limits the frequency of interspecific contact, although several species could achieve large effective population sizes.

The demographic differences between the genera might also lead to other speciation histories. The diversification of the *Branta*-clade was more gradual compared to the *Anser*-clade, which can be considered an adaptive radiation (Ottenburghs et al., 2016a). During an adaptive radiation the frequency of interspecific interactions increases, enhancing the probability of introgressive hybridization (Seehausen, 2004). Moreover, as the radiation progresses, occasional hybridization could facilitate further ecological diversification (Gilbert, 2003). Possibly, the diversification in beak morphology among *Anser* species was driven by hybridization, comparable to the radiation of Darwin's Finches on the Galapagos Islands (Lamichhaney et al., 2015, Almen et al., 2016).

8.4.4 A Hybrid Origin for the Red-breasted Goose?

The hybridization network analysis also suggested a possible alternative scenario in which the Red-breasted Goose is a hybrid species between the ancestors of the White-cheeked Geese and the Brent Goose. If so, the distinct morphology of this species might be the outcome of transgressive segregation (Rieseberg et al., 1999). But indisputably demonstrating hybrid speciation is challenging and often the most likely scenario for the observed genomic pattern is introgressive hybridization (Schumer et al., 2014). To our knowledge, five bird species have been proposed to have hybrid origins: the Italian Sparrow (*Passer italiae*, Hermansen et al., 2011), the Audubon's Warbler (*Setophaga auduboni*, Brelsford et al., 2011), the Genovesa Mockingbird (*Mimus parvulus bauri*, Nietlisbach et al., 2013), the Hawaiian Duck (*Anas wylvilliana*, Lavretsky et al., 2015b) and a recent lineage of Darwin's finches on Daphne Major (referred to as 'Big Bird', Grant and Grant, 2009). However, the hybrid origin of these putative cases has not been unequivocally established (Schumer et al., 2014). Also, in the case of the

Red-breasted Goose, the most parsimonious explanation seems to involve separate hybridization events between the Red-breasted Goose and the ancestors of the White-cheeked Geese and the Brent Goose.

8.5. Conclusion

Using genomic datasets and modern analytical tools, such as the D-statistic and PSMC analysis, in combination with network analyses based on gene tree discordance, we were able to determine patterns of introgressive hybridization in the True Geese. High levels of ancient gene suggest a scenario of divergence-with-gene-flow. We found indications for low levels of recent gene flow, but the quantification of this recent gene flow warrants a population genomic approach whereby multiple individuals per population are sequenced. The reconstruction of historical effective populations sizes indicate that most species showed a steady increase during the Pliocene and Pleistocene followed by population subdivision during the Last Glacial Maximum about 110,000 to 12,000 years ago. The combination of large effective population sizes and occasional range shifts might have facilitated contact between diverging goose species, resulting in the establishment of numerous hybrid zones and consequent gene flow. Our approach, based on genome-wide phylogenetic incongruence and network analyses, will be a useful procedure to reconstruct the complex evolutionary histories of many naturally hybridizing species groups.

8.6 Supplementary Material

Table S8.1. Significant D-statistics (|Z-score| > 3) for *Anser* and *Branta* species. A positive D-statistic and Z-score indicate gene flow between P2 and P3, while a negative D-statistic and Z-score indicate gene flow between P1 and P3.

P1	P2	P3	ABBA	BABA	D-statistic	Z-score
Barnacle Goose	Cackling Goose	Canada Goose	18937	15896	0.08728	4.32154
Barnacle Goose	Cackling Goose	Red-breasted Goose	16014	11925	0.14635	6.02174
Barnacle Goose	Cackling Goose	Black Brent Goose	14084	11674	0.09358	3.5156
Barnacle Goose	Canada Goose	Red-breasted Goose	15835	12952	0.10018	4.03718
Barnacle Goose	Hawaiian Goose	Red-breasted Goose	14972	12407	0.0937	4.19242
Barnacle Goose	Red-breasted Goose	Black Brent Goose	19676	17016	0.07249	3.02167
Greater White-fronted Goose	Lesser White-fronted Goose	Pink-footed Goose	13084	16863	-0.12618	-6.11657
Greater White-fronted Goose	Lesser White-fronted Goose	Swan Goose	17535	13932	0.11449	5.27904
Greater White-fronted Goose	Lesser White-fronted Goose	Greylag Goose	17125	13782	0.10818	5.26884
Greater White-fronted Goose	Lesser White-fronted Goose	Ross’ Goose	15993	12981	0.10396	5.32508
Greater White-fronted Goose	Lesser White-fronted Goose	Snow Goose	16712	11972	0.16526	7.33651
Greater White-fronted Goose	Tundra Bean Goose	Emperor Goose	11637	13631	-0.07891	-3.38305
Greater White-fronted Goose	Pink-footed Goose	Emperor Goose	11387	13590	-0.08824	-3.70132
Greater White-fronted Goose	Taiga Bean Goose	Emperor Goose	10929	14133	-0.12782	-5.45737
Greater White-fronted Goose	Taiga Bean Goose	Bar-headed Goose	11976	14156	-0.08341	-3.47019
Greater White-fronted Goose	Greylag Goose	Snow Goose	16277	14102	0.07162	3.05535
Greater White-fronted Goose	Greylag Goose	Ross’ Goose	16678	14016	0.0867	3.71949
Greater White-fronted Goose	Greylag Goose	Emperor Goose	13217	15482	-0.07889	-3.53742

Lesser White-fronted Goose	Tundra Bean Goose	Swan Goose	13220	18276	-0.16054	-8.14973
Lesser White-fronted Goose	Tundra Bean Goose	Greylag Goose	14000	17018	-0.0973	-4.64227
Lesser White-fronted Goose	Tundra Bean Goose	Snow Goose	12080	16580	-0.15702	-7.05339
Lesser White-fronted Goose	Tundra Bean Goose	Ross' Goose	12708	15639	-0.10336	-4.68194
Lesser White-fronted Goose	Tundra Bean Goose	Emperor Goose	12382	14254	-0.0703	-3.00986
Lesser White-fronted Goose	Pink-footed Goose	Swan Goose	13795	18656	-0.1498	-6.82075
Lesser White-fronted Goose	Pink-footed Goose	Greylag Goose	15205	17907	-0.08162	-3.5794
Lesser White-fronted Goose	Pink-footed Goose	Snow Goose	12656	17973	-0.017361	-7.03194
Lesser White-fronted Goose	Pink-footed Goose	Ross' Goose	13444	17102	-0.11974	-5.42133
Lesser White-fronted Goose	Pink-footed Goose	Emperor Goose	12726	14809	-0.07564	-3.16364
Lesser White-fronted Goose	Taiga Bean Goose	Swan Goose	12758	18105	-0.17324	-8.38472
Lesser White-fronted Goose	Taiga Bean Goose	Greylag Goose	14071	16576	-0.08177	-3.70011
Lesser White-fronted Goose	Taiga Bean Goose	Snow Goose	12152	15995	-0.13653	-6.22385
Lesser White-fronted Goose	Taiga Bean Goose	Ross' Goose	13053	15421	-0.08314	-3.93028
Lesser White-fronted Goose	Taiga Bean Goose	Emperor Goose	11307	14389	-0.11995	-5.41878
Lesser White-fronted Goose	Swan Goose	Greylag Goose	13629	16427	-0.09312	-4.06701
Lesser White-fronted Goose	Swan Goose	Snow Goose	11728	16056	-0.15579	-6.52462
Lesser White-fronted Goose	Swan Goose	Ross' Goose	12690	15630	-0.1038	-3.98444
Lesser White-fronted Goose	Greylag Goose	Snow Goose	13688	16353	-0.08565	-4.05429
Lesser White-fronted Goose	Greylag Goose	Emperor Goose	12720	14862	-0.07769	-3.47207
Tundra Bean Goose	Greylag Goose	Ross' Goose	16211	13632	0.08642	3.4077
Pink-footed Goose	Taiga Bean Goose	Greater White-fronted Goose	13230	15835	-0.08962	-4.41287
Pink-footed Goose	Taiga Bean Goose	Bar-headed Goose	11671	13749	-0.08173	-3.43567
Pink-footed Goose	Greylag Goose	Snow Goose	16514	13761	0.09092	3.41593
Pink-footed Goose	Greylag Goose	Ross' Goose	17046	13739	0.10741	4.49622
Greylag Goose	Swan Goose	Ross' Goose	14083	16672	-0.08417	-3.04915

Snow Goose	Emperor Goose	Lesser White-fronted Goose	14219	19017	-0.14437	-6.00712
Snow Goose	Emperor Goose	Taiga Bean Goose	12425	16463	-0.13976	-6.65546
Snow Goose	Emperor Goose	Tundra Bean Goose	13617	15787	-0.07382	-3.31701
Snow Goose	Emperor Goose	Greylag Goose	13575	17952	-0.13882	-5.59049
Ross' Goose	Emperor Goose	Lesser White-fronted Goose	14861	18236	-0.10199	-4.5106
Ross' Goose	Emperor Goose	Taiga Bean Goose	12663	16754	-0.13905	-6.34584
Ross' Goose	Emperor Goose	Tundra Bean Goose	13651	15969	-0.07827	-3.69219
Ross' Goose	Emperor Goose	Greylag Goose	13244	18412	-0.16325	-6.66969

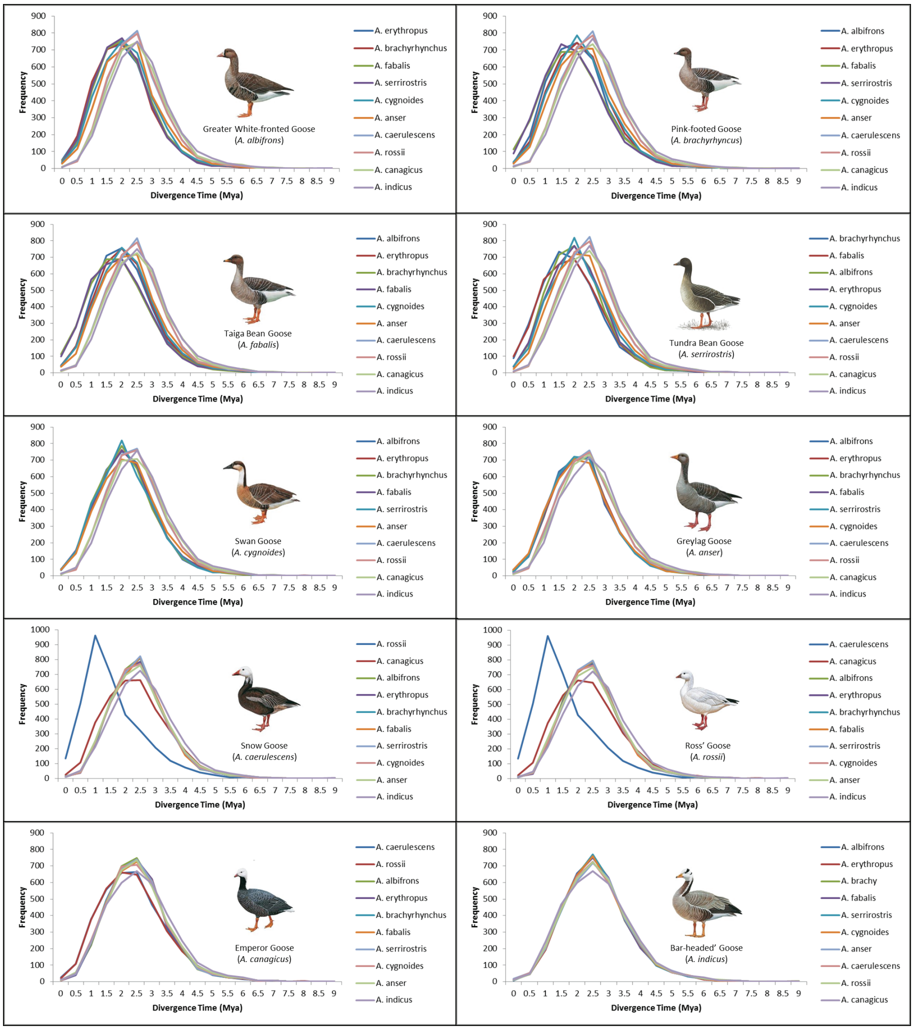


Figure S8.1 Distribution of gene tree divergence times for all goose species. All distributions show a single peak, indicating gene flow during divergence. The divergence time of several gene trees was close to zero, suggesting low levels of recent gene flow between certain species.

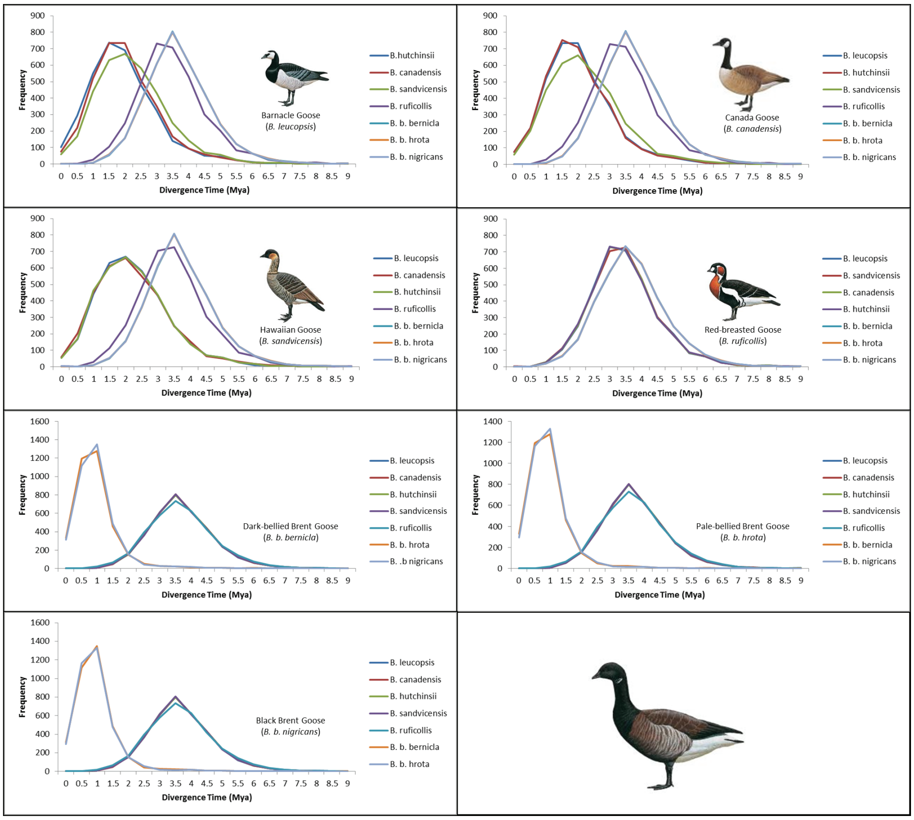


Figure S8.1 ***Continued.*** **Final three figures represent the three subspecies of Brent Goose, which is depicted in the lower right panel.**

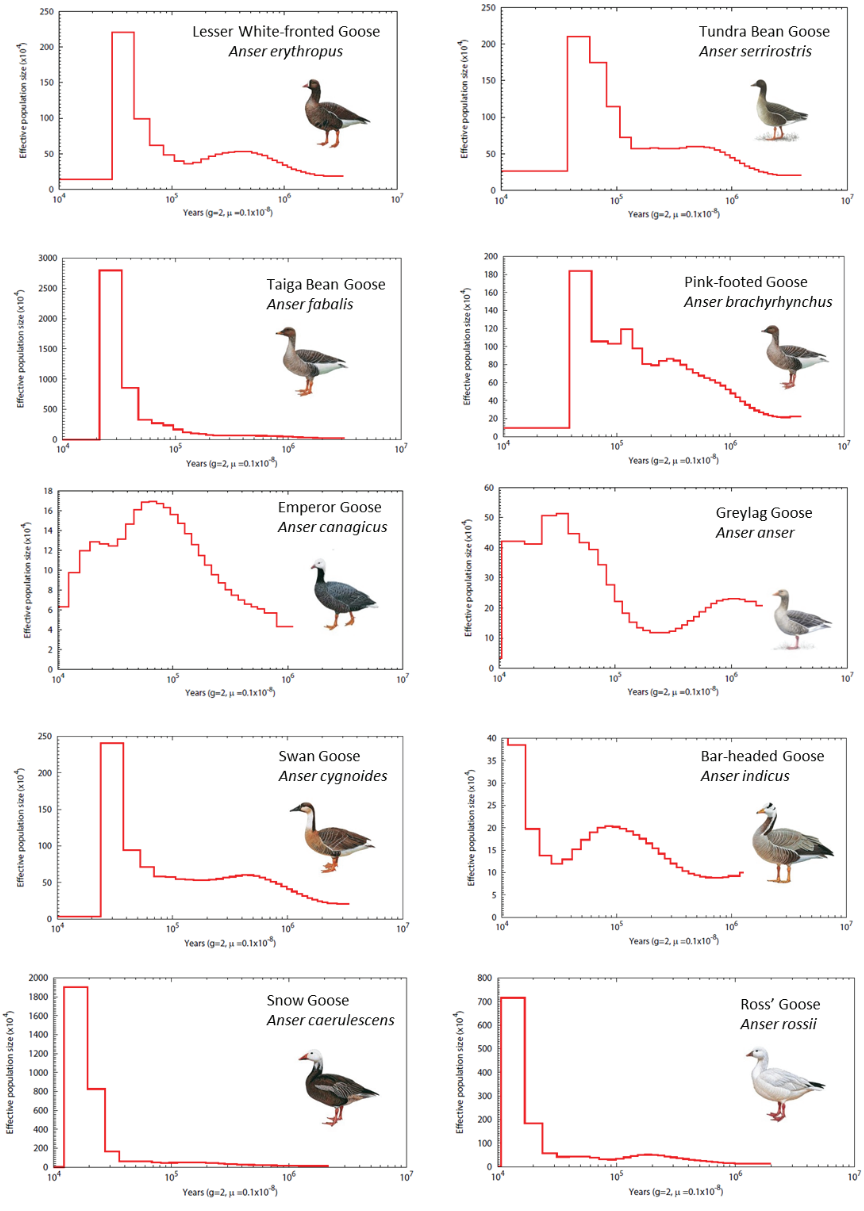


Figure S8.2 Estimates of historical effective population sizes for all goose species, based on a PSMC analysis.

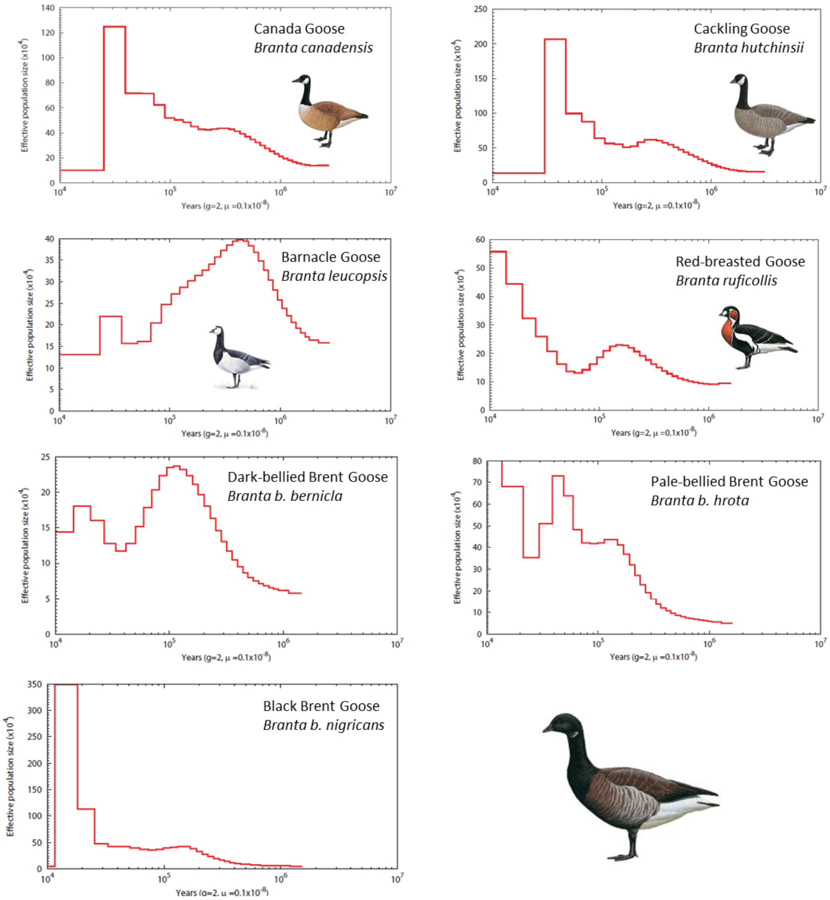


Figure S8.2 ***Continued.*** **Final three figures represent the three subspecies of Brent Goose, which is depicted in the lower right panel.**

Chapter 9

Synthesis: the syngameon scenario and the exploration of the adaptive landscape

9.1 Introduction

In this thesis I have highlighted the role of hybridization in avian evolution by quantifying the incidence of avian hybridization (Chapter 3) and by providing an overview of avian hybrid zone dynamics and consequent patterns of interspecific genetic exchange (Chapter 4). Moreover, the widespread occurrence of hybridization in birds might alter the way we depict evolutionary histories, shifting from a tree-based to a network approach (Chapter 5). With this general knowledge in mind, I explored the role of hybridization in the evolutionary history of the True Geese. First, I reviewed the current knowledge on hybrid geese, focusing on incidence and frequency of goose hybrids, behavioural mechanisms leading to hybridization and hybrid fertility (Chapter 6). Next, a phylogenetic framework was established (Chapter 7), which served as a basis to study patterns of interspecific gene flow among the different goose species (Chapter 8).

In this synthesis, I will combine the insights from these chapters into a novel way of interpreting evolution and species diversification; a viewpoint that I have dubbed the "Syngameon Scenario." First, I will outline this scenario with relevant examples and discuss the position of the True Geese in this scheme. Next, the Syngameon Scenario will serve as a travel guide when I consider how hybridization influences our views on phylogenetics, species concepts and adaptation.

9.2. The Syngameon Scenario

The concept of a syngameon, introduced by Lotsy (1925), has been used extensively in the botanic literature. In his book *Plant Speciation*, Grant (1981) described a syngameon as follows: "The definition of the biological species as the most inclusive breeding group does not hold up in cases of naturally hybridizing species and semispecies. Where limited gene exchange is taking place between otherwise isolated semispecies, the most inclusive unit of interbreeding is not a single biological species but an assemblage of semispecies. Such an assemblage is called a syngameon." The term syngameon has not become established in the scientific jargon: a keyword search for "syngameon*" in Thomson Reuters' Web of Science^{TM} resulted in only 22 papers (devoted to plants [12], corals [4], fish [2], butterflies [1], species concepts [2] and mating behaviour [1]). Despite its limited use in scientific publications, the syngameon concept can serve as an excellent framework to study the evolutionary history of naturally hybridizing species groups (Seehausen, 2004). In the following paragraphs, I will outline the Syngameon Scenario in three stages with relevant examples: (1) Formation of the syngameon, (2) Introgression and disintegration of the syngameon, and (3) End of the syngameon.

A syngameon can arise rapidly as an adaptive radiation or gradually as different species split off one by one while they remain genetically connected by gene flow (Stage One). If the formation of a syngameon is related to adaptive radiation, hybridization may be the driving factor of the radiation by generating variability and novel phenotypes (e.g., by transgressive segregation) for selection to act upon (Seehausen, 2004). Alternatively, hybridization might be a consequence of the rapid radiation if diversification was faster compared to the evolution of pre- and postzygotic isolation mechanisms (Wiens et al., 2006). These two scenarios are not always mutually exclusive: for example, ancient hybridization might be followed by rapid diversification which in turn leads to extensive hybridization among the resulting species. During the formation of a syngameon, the interacting species essentially form a single gene pool due to high levels of gene flow and can be seen as hybrid swarms (Arnold, 1997, Li et al., 2016b, Wiens et al., 2006).

After the initial stage of syngameon formation, the build-up of pre- and postzygotic isolation mechanisms results in the disintegration of the syngameon into reproductively isolated and independently evolving lineages. The nature of these isolation mechanisms can be depicted on the speciation continuum as discussed in Chapter 2. It is important to keep in mind that movement of species along the speciation continuum is not constant; speciation can go back and forth at different speeds or come to a halt at certain stages (e.g., formation of a stable hybrid zone). Movement across the speciation continuum during Stage Two of the Syngameon Scenario can thus result in merge-and-diverge (or fission-fusion) dynamics in which species oscillate between periods of divergence under selection and convergence due to introgressive hybridization under the influence of ecological conditions and the nature of reproductive isolation (Grant and Grant, 2008). Moreover, the isolation mechanisms need not be the same between different species in the syngameon (Coyne and Orr, 1989, Coyne and Orr, 1997). For instance, in the *Drosophila melanogaster* group, the subspecies *biauraria* and *triauraria* are partially reproductively isolated by prezygotic isolation, whereas reproductive isolation between *triauraria* and *quadraria* is largely the outcome of postzygotic isolation mechanisms (Coyne and Orr, 1997). As long as reproductive isolation is incomplete and the syngameon has not fully disintegrated, occasional hybridization might lead to interspecific gene flow. Studies on hybrid zones show that the patterns of introgression vary considerably depending on numerous factors (Chapter 4). Stage Two can be regarded as the introgression/disintegration-stage in the Syngameon Scenario.

The syngameon ends when all interacting species reach complete reproductive isolation, resulting in the termination of gene flow (Stage Three). This complete reproductive isolation might be the outcome of strong ecological or sexual selection against partially fertile hybrids or intrinsic postzygotic isolation mechanisms leading to sterile or inviable hybrids (Coyne and Orr, 2004). The rate at which postzygotic isolation mechanisms evolve differs between taxonomic groups (Fitzpatrick, 2004, Prager and Wilson, 1975): for example, hybrid inviability evolves after on average 4 and 21 million years in mammals and birds, respectively (Fitzpatrick, 2004).

9.2.1 The Syngameon Scenario as a Study System

Studying a group of hybridizing species within the Syngameon Scenario involves three steps: (1) quantify the incidence and frequency of hybridizing species, (2) determine the patterns of ancient and recent gene flow and (3) characterize pre- and postzygotic isolation mechanisms between the interacting species. The first step indicates how many species are potentially part (or have been part) of the syngameon. The second step shows which species are exchanging or have exchanged genetic material, thereby delineating the actual syngameon. Finally, the third step places the interacting species on the speciation continuum.

Step 1: Detecting Putative Syngameons by Hybrid Networks

Most studies on avian hybridization have focused on the interaction of two species, mostly in the context of hybrid zones (Price, 2008). However, the widespread occurrence of hybridization in birds (Chapter 3) and the diversity of introgression patterns in avian hybrid zones (Chapter 4) indicate that avian evolution should be studied with the Syngameon Scenario in mind. Clear examples of avian syngameons include Darwin's Finches (Lamichhaney et al., 2015), *Larus* Gulls (Sonsthagen et al., 2012), *Anas* Ducks (Kraus et al., 2012, Lavretsky et al., 2014) and North American Wood Warblers of the Parulidae family (Willis et al., 2014). The widespread occurrence of avian hybridization suggests that there are many more syngameons. Based on the records retrieved from the Serge Dumont Bird Hybrids Database (Dumont, 2014), I constructed networks to visualize, quantify and compare the hybrid interactions within certain bird orders and families (Figure 9.1 and 9.2). These "hybrid networks" provide the first step in characterizing putative syngameons. In this case, hybrid networks only capture the incidence of hybridization, indicating whether a certain combination has been observed or not, but they could convey more information regarding the nature of certain combinations, such as the frequency or the fertility of hybrids.

Analyses of these networks can identify certain "hub-species" that interbreed with numerous other species. In case of the Galliformes, the Common Pheasant (*Phasianus colchicus*) is connected with 14 other species, including species from the subfamilies Tetraoninae (Grouse) and Meleagridinae (Turkeys). The numerous hybrid interactions of this species can be explained by human-mediated introductions across the globe (Drake, 2006). A similar hub-species in the Order Anseriformes is the Mallard (*Anas platyrhynchos*), which has hybridized with at least 39 different species (Figure 9.2). In contrast to the Common Pheasant, several of the hybrid interactions of the Mallard have been studied in greater detail, specifically with closely related species, such as the Black Duck (*Anas superciliosa*) in Australia and New Zealand (Taysom et al., 2014), the Hawaiian Duck (*Anas wylvilliana*) on the Hawaiian Islands (Fowler et al., 2009), the American Black Duck (*Anas rubripes*) and the Mottled Duck (*Anas fulvigula*) in North America (Mank et al., 2004, Peters et al., 2014a), the Spot-billed Duck (*Anas zonorhyncha*) in Russia (Kulikova et al., 2004) and the Mexican Duck (*Anas diazi*) in Mexico (Lavretsky et al., 2015a).

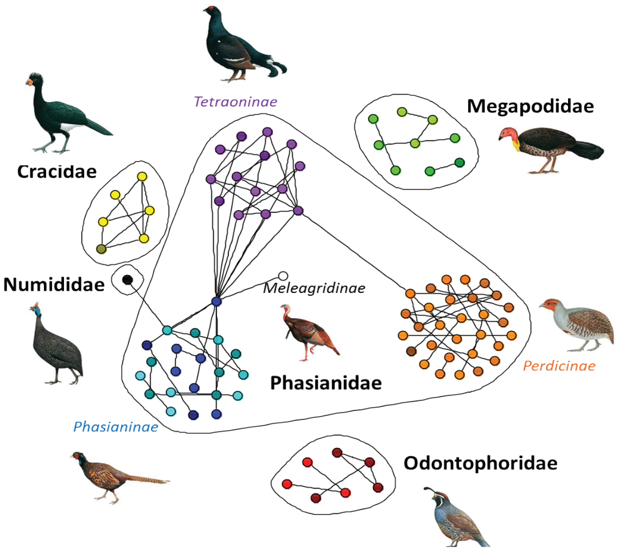


Figure 9.1. A network depicting hybrid interactions between different species (depicted as dots) in the order Galliformes. Different colours represent different (sub)families and different shades represent different genera. Drawings used with permission of Handbook of Birds of the World (del Hoyo and Elliott, 1992).

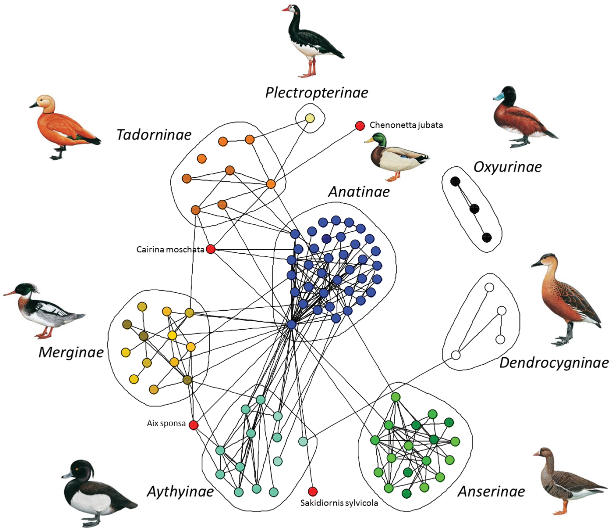


Figure 9.2. A network depicting hybrid interactions between different species (depicted as dots) in the order Anseriformes. Different colours represent different subfamilies and different shades represent different genera. Red dots are species with a unclear taxonomic position. Drawings used with permission of Handbook of Birds of the World (del Hoyo and Elliott, 1992).

Step 2: Quantifying Gene Flow

Based on the hybrid network, hypotheses regarding recent and ancient gene flow can be formulated and tested for particular species pairs. For example, a high frequency of hybrids and backcrosses might suggest high levels of recent gene flow, whereas the occurrence of sterile hybrids might be a remnant of an old syngameon, suggesting ancient gene flow. The recent developments in avian genomics offer opportunities to quantify levels of recent and ancient gene flow (Kraus and Wink, 2015, Jarvis, 2016, Toews et al., 2016) and to reconstruct the evolutionary history of a syngameon. For example, many studies have quantified the amount of recent gene flow between certain species pairs in the genus *Anas* (McCracken and Wilson, 2011, Joseph et al., 2009, Guay et al., 2015, and see studies on Mallard hybridization listed above) and several studies suggested high levels of ancient gene flow in this genus (Kraus et al., 2012, Lavretsky et al., 2014, Peters et al., 2014b).

Step 3: Reproductive Isolation

The amount of recent gene flow provides an indication of the degree of reproductive isolation between certain species. Determining which reproductive isolation mechanisms (see Chapter 2 for an overview) prevent or limit gene flow can be accomplished by experiments, field observations and genomic analyses. The interplay of different reproductive isolation mechanisms can be visualised on the speciation continuum (Seehausen et al., 2014). Speciation can be driven by divergent sexual or ecological selection, in which case extrinsic postzygotic and prezygotic mechanisms act first and intrinsic postzygotic mechanisms evolve later in the speciation process. Alternatively, speciation can be driven by intrinsic postzygotic mechanisms while extrinsic postzygotic and prezygotic mechanisms can accumulate and reinforce reproductive isolation at a later stage. Hendry et al. (2009) recognized four stages across the speciation continuum: (1) continuous variation without reproductive isolation, (2) discontinuous variation with minor reproductive isolation, (3) strong, but reversible, reproductive isolation, and (4) strong and irreversible reproductive isolation. Complementing the speciation continuum, the entire speciation process can be depicted in the speciation cubes, as described in Chapter 2.

9.2.2. A Syngameon Scenario for the True Geese

In Chapters 6, 7 and 8, I explored the role of hybridization in the evolutionary history of the True Geese. In this section, I will discuss these findings in the framework of the Syngameon Scenario, following the three steps outlined above.

Using the data on natural and captive hybridization from Chapter 6, I constructed a hybrid network for the True Geese (Figure 9.3). This network shows that all goose species are currently connected by hybridization. Hub-species include Barnacle Goose (hybrids with 14 other species), Greater White-fronted Goose (13), Snow Goose (13) and Canada Goose (13). Hybrids involving Hawaiian Goose have only been documented with one species (Swan Goose) in captivity (McCarthy, 2006).

The hybrid network thus shows that all goose species are currently connected by hybridization. But this does not imply that all these species are also connected or have been connected by gene flow. For example, extensive hybridization between black stilt (*Himantopus novaezelandiae*) and pied stilt (*Himantopus himantopus*) in New Zealand did not result in introgression, which was partly attributed to reduced hybrid fitness (Steeves et al., 2010). With regard to the True Geese, the D-statistic analyses from Chapter 8 suggested extensive ancient gene flow between different species. For the genus *Branta* putative ancient hybridization events (e.g., between Red-breasted Goose and the ancestor of the White-cheeked Geese) could be reconstructed, while the hybrid interactions in the genus *Anser* proved to be too complex to be confidently reconstructed. There were indications for low levels of recent gene flow, but this remains to be confirmed and quantified by a population genomic approach.

The fertility pattern reported in Chapter 6 showed that female hybrids are mostly sterile at high genetic distances, while male hybrids are always fertile. This pattern indicates intrinsic postzygotic isolation mechanisms between distantly related species. Hybrids between closely related species, on the other hand, are fertile, suggesting that species boundaries are mainly preserved by prezygotic and/or extrinsic postzygotic isolation mechanisms. It thus seems that the establishment and preservation of species boundaries in the True Geese is mainly driven by prezygotic and/or extrinsic postzygotic isolation mechanisms and that intrinsic postzygotic isolation mechanisms can be expected to evolve later. Hence, the True Geese occupy a similar position as *Heliconius* butterflies on the speciation continuum (Figure 9.4). This position corresponds to stage three in the classification of Hendry et al. (2009), namely strong, but reversible, reproductive isolation.

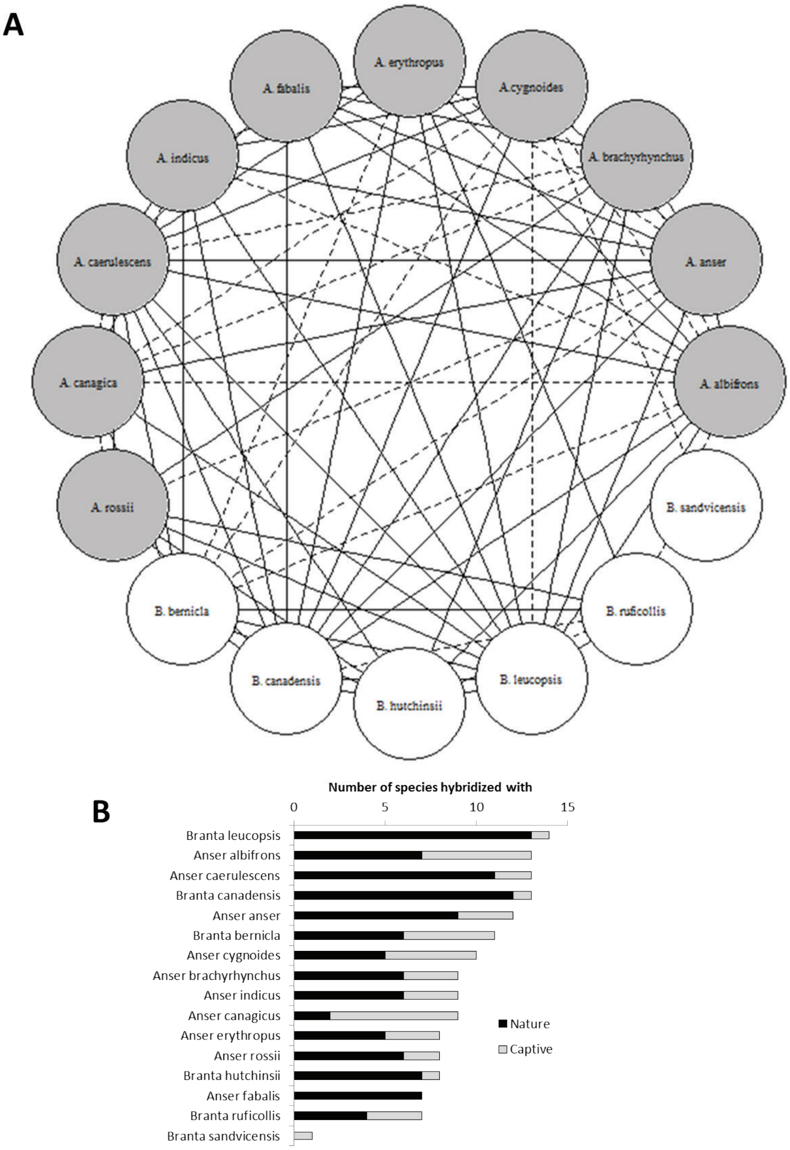


Figure 9.3. Patterns of hybridization in the True Geese. (A) Hybrid network with grey (*Anser*) and white circles (*Branta*) representing the different species. Full lines indicate natural hybridization, while dotted lines indicate captive hybridization. (B) Incidence of natural and captive hybridization for each species.

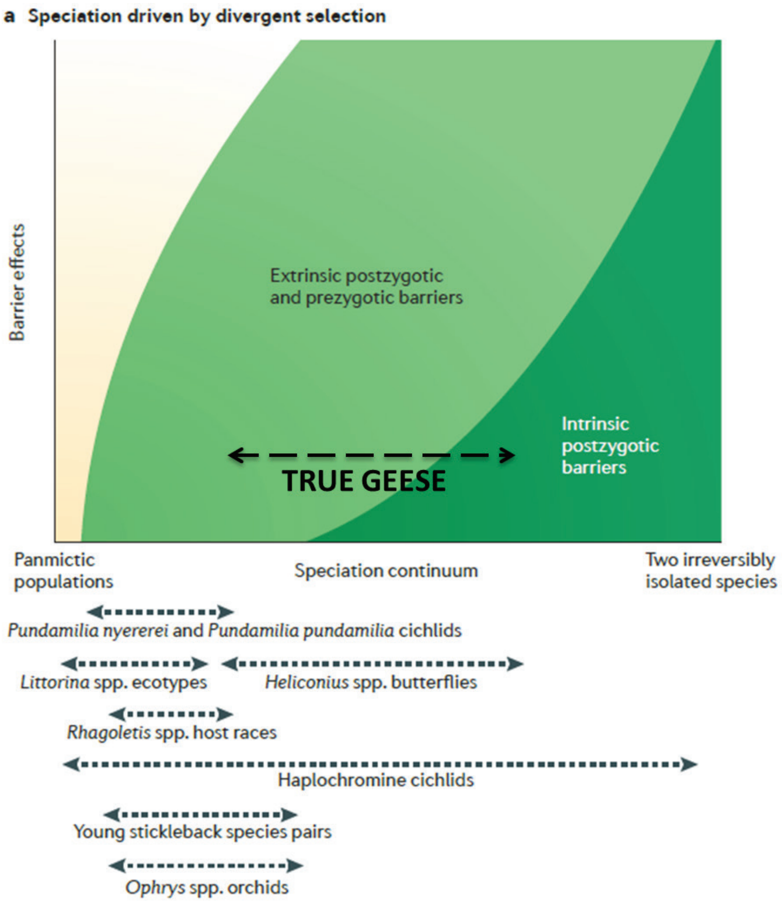


Figure 9.4 The position of the True Geese on the speciation continuum. The x-axis depicts the position of a diverging taxon pair on the speciation continuum and the y-axis represents the strength of reproductive isolation. Shapes of the curves are hypothetical. The establishment and preservation of species boundaries in geese is mainly driven by prezygotic and/or extrinsic postzygotic isolation, intrinsic postzygotic isolation mechanisms evolve later.

The analyses of gene flow and their position on the speciation continuum suggest that the diversification of True Geese may have been influenced by reinforcement, i.e. "the process, in which traits that increase prezygotic isolation between two differentiated populations are favoured due to natural selection against the production of unfit hybrids or otherwise maladaptive interbreeding" (Saetre, 2012). The exact selective pressures against hybrids remain to be determined by experiments and field observations. One possibility concerns the colour of the tail-coverts: all goose species have white tail-coverts, whereas those of hybrids are often barred or dusky (Gustavsson, 2010). This morphological disparity might hamper mate choice because these aberrant tail-coverts could lower the attractiveness of the hybrids (perhaps by giving them a juvenile appearance).

Based on the knowledge on goose hybridization and speciation, I can now sketch a possible Syngameon Scenario for the True Geese. Present-day goose species can be regarded as two related syngameons (corresponding to the genera *Anser* and *Branta*) that originated in contrasting ways. The *Anser*-syngameon is the result of an adaptive radiation, while the *Branta*-syngameon gradually expanded as different species split off one by one while they remained genetically connected by gene flow. Whether these genera are (or have been connected) by gene flow remains to be investigated, although the fertility patterns from Chapter 6 indicate that gene flow is possible through fertile male hybrids. This would suggest that the True Geese can be considered as one syngameon. After the formation of these syngameons, prezygotic isolation mechanisms limited gene flow and increased species divergence, possibly reinforced by selection against hybrids due to extrinsic and/or intrinsic postzygotic isolation mechanisms. During their diversification, several goose species remained connected by occasional hybridization, mediated by population expansion and possibly range shifts during the climatic oscillations of the Pleistocene (Chapter 7 and 8).

9.3. Phylogenetic Networks and the Notion of Species

As advocated in Chapter 5, the widespread occurrence of introgression in birds, in combination with the pervasiveness of incomplete lineage sorting, warrants a phylogenetic network approach to depict and analyse complex evolutionary histories. Although the algorithms to construct phylogenetic networks have not yet reached the complexity of phylogenetic tree-building methods, promising tools are being developed (Huson et al., 2010, Hejase and Liu, 2016). For instance, the Multispecies Network Coalescent (MSNC) expands the multispecies coalescent to take into account hybridization and incomplete lineage sorting to infer species trees (Wen et al., 2016a, Wen et al., 2016b).

The usefulness of phylogenetic networks is particularly apparent in prokaryotes where horizontal gene transfer swamps tree-like signals (Andam et al., 2010, Gogarten and Townsend, 2005, Philippe and Douady, 2003), prompting many microbiologists to argue that the phylogeny of microbial life resembles a web or a ring (Doolittle, 1999, Rivera and Lake, 2004, McInerney et al., 2014, Puigbo et al., 2009). Woese (2002) stated that there is a "genealogy-defining core set of genes" in spite of high levels of horizontal gene transfer and he suggested that co-adaptation between these key genes would push populations over a "Darwinian threshold", leading to divergence into separate bacterial species. However, it became clear that horizontal gene transfer severely hampers the construction of phylogenetic trees (Creevey et al., 2011, Dagan and Martin, 2006), questioning the existence of a "genealogy-defining core set of genes" in prokaryote evolution.

The advent of genomic data in phylogenetics showed that the occurrence of phylogenetic incongruence (i.e. analyses of different genes resulting in discordant gene trees) is a common and widespread phenomenon in eukaryotes as well (Rokas et al., 2003). In contrast to prokaryotes, this phylogenetic incongruence is less problematic and tree-like patterns are readily discernible in eukaryote phylogenies. This was also apparent in the phylogenomic analyses of the True Geese in Chapter 7: despite high levels of incomplete lineage sorting and hybridization, both the concatenation and the consensus method resulted in identical topologies. These findings indicate the existence of a dominant species history, possibly supported by an underlying "genealogy-defining core set of genes."

Hence, Woese's (2002) concept of a "genealogy-defining core set of genes" could be applied to the species problem. In Chapter 2, an analysis of the species problem indicated that species are "an entity composed of organisms which maintains its identity from other such entities through time and over space, and which has its own independent evolutionary fate and historical tendencies" (Wiley and Mayden, 1997). But how can taxonomists identify such independently evolving entities when hybridization occurs? It should be possible to identify a set of genes that keeps species reproductively isolated from other species despite hybridization. The Syngameon Scenario indicates that different species are reproductively isolated by different mechanisms. Hence, it can be expected that this core set of genes will depend on the nature and stage of the speciation process, which is in line with the "life history" approach to the species problem (Harrison, 1998).

This core set of genes can be identified by studying patterns of introgression: a subset of the genome that confers reproductive barriers between species is expected to exhibit reduced introgression (Payseur, 2010). For example, genomic analyses of the hybridizing monkeyflowers *Mimulus guttatus* and *Mimulus nasutus* uncovered reduced introgression in several genomic regions, including one region that contains genes related to flowering phenology and thus temporal reproductive isolation (Kenney and Sweigart, 2016). Similarly, the X-chromosome exhibits lower introgression rates compared to autosomes across the hybrid zone between house mouse (*Mus musculus*) subspecies (Macholan et al., 2007, Teeter et al., 2008, Teeter et al., 2010), suggesting a role for genes on the X-chromosome in male hybrid sterility (Good et al., 2008, Storchova et al., 2004).

Which genes contribute to reproductive isolation between the different goose species remains to be investigated. The hybrid zone between Canada Goose and Cackling Goose (Leafloor et al., 2013) provides an excellent system to test whether it is possible to characterize a core set of genes that contribute to reproductive isolation between these goose species. For the other hybridizing goose species, a comparative population genomic approach can be applied to identify putative sets of core genes. The high levels of phylogenetic incongruence reported in Chapter 7 suggest that different genes might be involved in the reproductive isolation of different goose species.

9.4. Exploring the Adaptive Landscape

In Chapter 7, I showed that the Grey Geese can be regarded as an adaptive radiation in terms of beak morphology following the criteria of Schluter (2000). Although less pronounced, the other groups of goose species (the White Geese and the Black Geese) also display a variety of beak morphologies. Different goose species seem to be adapted to different ecological feeding niches. For instance, the Taiga Bean Goose has a long thin bill adapted for probing soft substrates of bogs and marshlands to feed on underground plant material, whereas the Pink-footed Goose has a short bill suitable for grazing and seed-stripping (Owen, 1976, Reed, 1976).

In Chapter 8, I suggested that introgressive hybridization might have facilitated the diversification in terms of beak morphology in the True Geese. Can hybridization facilitate adaptation in certain traits? And how does the adaptive potential change during different stages of the Syngameon Scenario? To answer these questions, I will develop a conceptual mathematical model based on the adaptive landscape metaphor and the Syngameon Scenario. But first I need to provide the proper context because the adaptive landscape metaphor has been used in many different ways.

9.4.1 A Menagerie of Adaptive Landscapes

The adaptive landscape metaphor was introduced by Sewall Wright (1932) at the Sixth International Congress of Genetics in Ithaca, New York (although Mccoy (1979) argued that the French engineer Armand Janet already presented the idea in 1895 at a zoology conference in Leiden). This metaphor was a way for Wright to convey his mathematically complex shifting balance theory of evolution (Wright, 1931). The foundation of the adaptive landscape is a multidimensional space of genotype combinations (Dobzhansky (1937) estimated that this space would contain at least 10^{1000} different combinations). Each of these combinations has a certain fitness value which is represented on a two-dimensional landscape: high fitness values are depicted as peaks, while low fitness values result in valleys (Figure 9.5A).

Wright's original adaptive landscape has been criticized as mathematically incoherent (Coyne et al., 1997, Provine, 1989) and some have suggested to abandon the adaptive landscape metaphor altogether (Kaplan, 2008, Pigliucci, 2008), whereas others emphasize its heuristic value (Skipper, 2004, Plutynski, 2008). The heuristic value of the adaptive landscape metaphor is apparent in the way other biologists have applied it to various situations. In his book *Genetics and the Origin of Species*, Dobzhansky (1937) used the adaptive landscape to explain the genetic underpinnings of evolution under selection, drift, mutation and migration (Figure 9.5B). Simpson (1944) combined the adaptive landscape with palaeontology and introduced the phenotypic landscape in order to explain the evolution of horses in *Tempo and Mode in Evolution* (Figure 9.5C). And Maynard Smith (1970) applied the adaptive landscape to explore the space of possible protein sequences. These three lineages of adaptive landscapes (referred to as genetic, phenotypic and molecular landscapes, respectively) have

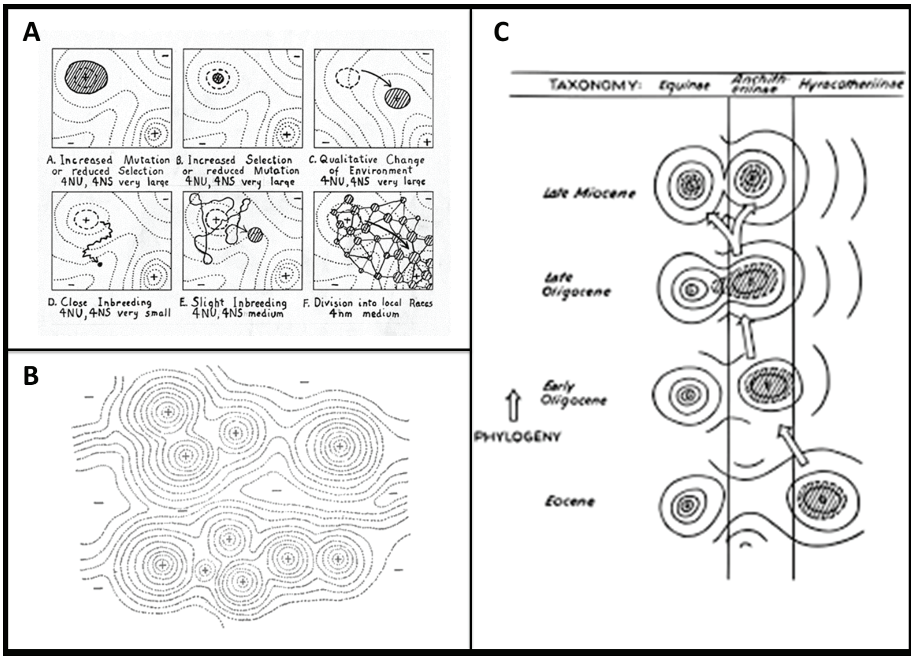


Figure 9.5. Three applications of the adaptive landscape metaphor. (A) The original formulation by Wright (1932) to explain the shifting balance theory of evolution. (B) Example of an adaptive landscape in *Genetics and the Origin of Species* by Dobzhansky (1937) in order to clarify the genetic underpinnings of evolution under drift, mutation and migration. (C) Phenotypic landscape of equine evolution in *Tempo and Mode of Evolution* by Simpson (1944).

led to a wide usage of this metaphor, including the development of several mathematical applications, such as theoretical morphospace (Raup, 1967, Raup, 1966), fitness surfaces (Lewontin and Kojima, 1960, Lewontin and White, 1960, Lande and Arnold, 1983, Lande, 1976) and holey landscapes (Gavrilets, 1997, Gavrilets, 1999, Gavrilets, 2004).

The genetic landscape introduced by Wright (1932) and popularized by Dobzhansky (1937) was a depiction of evolutionary possibilities in an imaginary genotype space. Richard Lewontin was the first to construct an adaptive landscape based on empirical genetic data and fitness estimates. He created a fitness landscape based on the frequencies of two chromosomal inversions in grasshopper (*Moraba scurra*) populations and their fitness values (Lewontin and Kojima, 1960, Lewontin and White, 1960). Similarly, the phenotypic landscapes (as introduced by Simpson, 1944) can be constructed on the basis of empirical data (Fear and Price, 1998), as illustrated by a classic study on Red Crossbills (Benkman, 2003).

Red Crossbills (*Loxia curvirostra* complex) represent a recent radiation that can be divided into nine distinct 'call-types' (Benkman, 1999, Groth, 1993), of which at least seven are specialized for foraging on different conifer species (Parchman and Benkman, 2002, Benkman, 1999, Benkman et al., 2001). Bill depth and palate groove width determine feeding rate, which in turn affects individual survival (a good proxy for fitness; Crone, 2001). Benkman (2003) constructed a fitness surface for Red Crossbills with bill depth and palate groove on the axes and survival probability as the height of the surface. This surface displays distinct peaks and valleys (i.e. a rugged landscape) with each crossbill call-type residing close or near the summits (Figure 9.6). Hence, each call-type seems to inhabit a distinct ecological feeding niche.

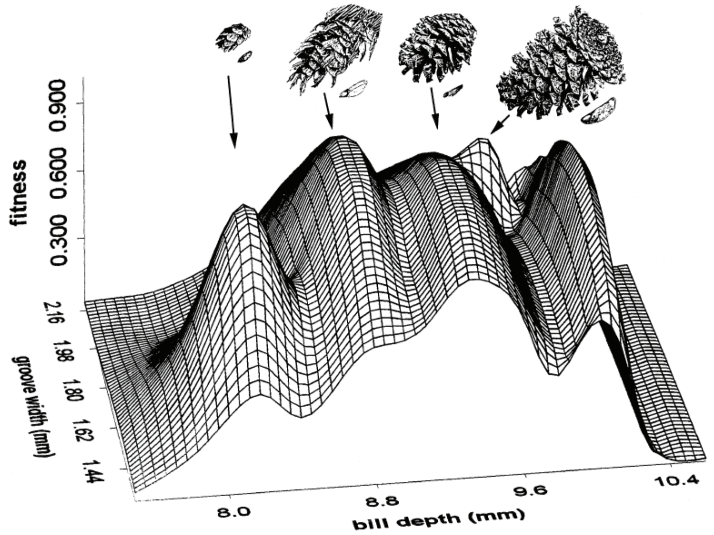


Figure 9.6. Fitness surface for five Red Crossbill call-types based on foraging efficiency and survival data. The peaks correspond to particular conifer species (from left to right: western hemlock, Douglas fir, Rocky Mountains lodgepole pine, ponderosa pine and South Hills lodgepole pine) – Adapted from Benkman (2003).

9.4.2 Hybridization and Adaptation

The capacity of a population to adapt to novel environmental conditions, i.e. adaptive potential or evolvability (Willi et al., 2006, Houle, 1992), is driven by ecological opportunities and the available genetic and phenotypic variation (Grant and Grant, 2011). Ecological opportunity concerns "environmental conditions that both permit the persistence of a lineage within a community, as well as generate divergent natural selection within that lineage" (Wellborn and Langerhans, 2015) and can be interpreted as the number of empty, unexplored peaks in an adaptive landscape (Erwin, 2015, Wellborn and Langerhans, 2015, Yoder et al., 2010) or empty ecological niches. The available genetic and phenotypic variation is provided by standing genetic variation, *de novo* mutations, phenotypic plasticity and introgressive hybridization.

The classic view of adaptation is that natural selection operates on new mutations or standing genetic variation (Barrett and Schluter, 2008, Orr, 2005). Hybridization can facilitate adaptive evolution by transferring advantageous alleles across species boundaries (Anderson and Stebbins, 1954, Lewontin and Birch, 1966, Hedrick, 2013, Arnold et al., 2008), by increasing standing genetic variation (Grant and Grant, 2010, Hedrick, 2013), or by creating novel phenotypes by transgressive segregation (Rieseberg et al., 1999, Rieseberg et al., 2003, Stelkens and Seehausen, 2009). Through these processes, hybridization might enable populations to quickly adapt to changing environments (Hedrick, 2013, Hamilton and Miller, 2016, Hoffmann and Sgro, 2011).

The contribution of introgressive hybridization to the adaptive potential of a population is expected to vary across different stages of the Syngameon Scenario, influenced by the nature of reproductive isolation. To gain more insights into the role of hybridization in adaptation within the Syngameon Scenario, I developed a conceptual mathematical model to simulate the exploration of phenotypic adaptive landscapes. The goal of the model is thus to assess the impact of hybridization on adaptive evolution under different levels of pre- and postzygotic isolation.

Consider an adaptive landscape with two uncorrelated phenotypic traits, each ranging from 0 to 100, containing ten adaptive peaks randomly placed on the landscape. The simulated adaptive landscape is assumed to be static. In reality, however, an adaptive landscape might be dynamic due to environmental changes and the actions of the organisms themselves (Laughlin and Messier, 2015, Jones et al., 2004). The adaptive peaks follow an isotropic bivariate Gaussian distribution with a standard height of 1 (fitness, F) and a width determined by the standard deviation (SD). The remainder of the adaptive landscape, outside of the ranges of the peaks, has a fitness value of 0. A starting trait-combination is randomly placed on this landscape, provided that the fitness is not 0, and evolution is simulated. With a probability p the offspring (O) of the current trait-combination (Parent1, P_1) is the result of hybridization. In such a case, a second parent (Parent2, P_2) is chosen randomly from anywhere in the landscape and the offspring's phenotype is positioned halfway P_1 and P_2. The random selection of P_2 reflects the Syngameon Scenario in which multiple species are interacting. If one would model the exploration of the adaptive landscape by two hybridizing species, the selection of P_2 would be restricted to a particular space in the adaptive landscape, corresponding to the phenotypic variation of this species. The assumption of an intermediate hybrid phenotype is based on the observation that most hybrids do display intermediate phenotypes compared to their parental taxa (Stelkens and Seehausen, 2009). Alternatively, with probability 1-p the offspring is not the result of hybridization, but generated using an isotropic bivariate Gaussian distribution centred on P_1, assuming that the phenotypic trait has a high heritability, such as beak depth in Darwin's finches (Grant and Grant, 2011). In any case (whether it is a hybrid or not): the offspring's phenotype is rejected when the fitness corresponding to its phenotype is 0. The offspring's phenotype is accepted when its fitness is higher than that of P_1: $F(O) > F(P_1)$. When the offspring's fitness is higher than 0, but smaller than $F(P_1)$: it is accepted with a probability: $F(O)/F(P_1)$. This takes into account the possibility that relatively unfit hybrids might occasionally be able to survive and reproduce (Arnold and Hodges, 1995). In case the offspring's phenotype is accepted, evolution continues, the offspring becomes P_1 and the process repeats. When the offspring's phenotype is rejected, the process is repeated until there is an offspring's phenotype that is accepted, and then evolution continues from there. This process of evolution is repeated for t generations.

The effect of hybridization on the exploration of the phenotypic adaptive landscape is thus modelled by two parameters: the percentage of hybrids (p) and selection against hybrids (SD of peaks). The percentage of hybrids indicates what the chance is that a hybrid is produced at every time step and can be regarded as the strength of prezygotic isolation. Selection against hybrids is determined by the width of the adaptive peaks in the landscape: wider peaks lead to a more uniform landscape increasing the chance that the fitness of the hybrid is higher than or equal to that of the parent. Narrow peaks, on the other hand, create a largely flat, low fitness landscape, thereby decreasing the chance that hybrid fitness equals or exceeds parental fitness. The parameter of peak width can thus be regarded as a measure for postzygotic isolation.

The exploration of the adaptive landscape is quantified by the number of peaks discovered by the population (i.e. exploration efficiency). It is expected that hybridization will increase the efficiency with which a population explores the landscape, because the phenotype of hybrids mostly lies outside the range of parental P_1 phenotype, thereby reaching unexplored territory on the adaptive landscape. The effect of hybridization on this exploration efficiency depends on the strength of prezygotic (percentage of hybrids) and postzygotic (peak width) isolation. I expect that postzygotic isolation will have a stronger effect on the exploration of the adaptive landscape compared to prezygotic isolation, because it is not the amount of hybrids but the amount of successful hybrids that determines the exploration efficiency.

The model was run in R version 3.2.2 with different combination of the parameters discussed above: percentage of hybrids (ranging from 0 to 20%) and the width of the peaks (ranging from 0 to 10 SD). Figure 9.7 gives an overview of an example run with five peaks and nine parameter combinations. In the final model the adaptive landscape contained 10 peaks, every parameter combination was run for 100 iterations and every iteration was comprised of 1000 time steps.

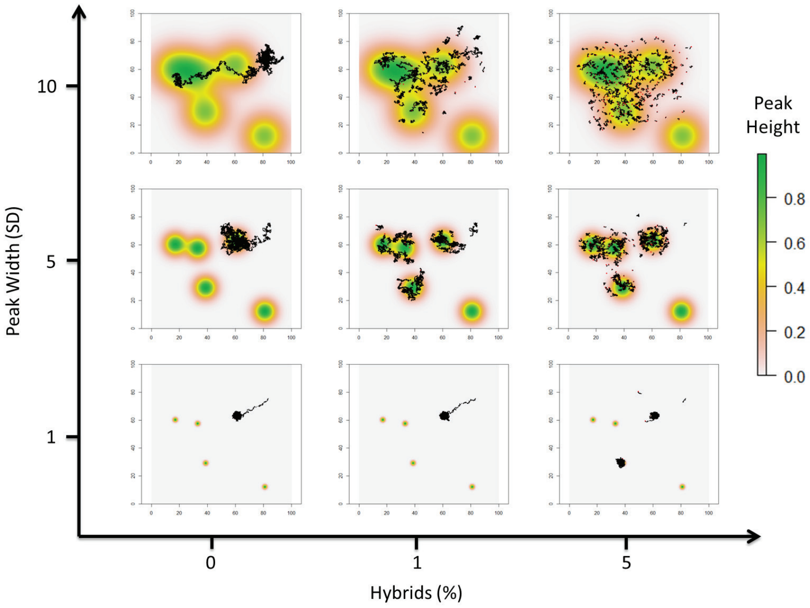


Figure 9.7. Example run of the model with 5 adaptive peaks and three values for peak width (1, 5 and 10 SD) and percentage of hybrids (0, 1 and 5%). Red to green gradient represents height of the peak (fitness). Pure (black dots) and hybrid (red dots) individuals explore the landscape. With increasing peak width and percentage of hybrids more peaks in the landscape are found.

The results from the model (Figure 9.8) indicate that hybridization can indeed have a positive effect on the exploration of an adaptive landscape: with an increasing percentage of hybrids, more adaptive peaks are found. However, the effect of hybridization on exploration efficiency depends heavily on the fitness of the hybrids which is determined by postzygotic isolation. In a landscape with wide peaks (i.e. weak postzygotic isolation) more adaptive peaks will be discovered compared to a landscape with narrow peaks (i.e. strong postzygotic isolation).

Within the Syngameon Scenario, the adaptive potential is expected to be highest during the formation of the syngameon when there is little or no reproductive isolation. As species diverge genetically, the build-up of reproductive isolation will limit the production and fitness of hybrids, thereby decreasing the adaptive potential of species within the syngameon. The nature of reproductive isolation mechanisms will determine the rate at which adaptive potential diminishes during species divergence. If species are largely reproductively isolated by prezygotic isolation mechanisms, they might still be able to discover new adaptive peaks because of weak postzygotic selection against occasional hybrids. On the other hand, if reproductive isolation is the outcome of postzygotic isolation mechanisms, strong selection against hybrids will hinder the exploration of the adaptive landscape.

Avian speciation is largely driven by prezygotic isolation mechanisms (Grant and Grant, 1997, Price, 2008) and intrinsic postzygotic isolation evolves slowly in birds (Price and Bouvier, 2002, Prager and Wilson, 1975). These general characteristics of the avian speciation process suggest that hybridization could be a common and integral component of adaptive evolution in birds. Indeed, based on the long term ecological studies on Darwin’s Finches, Grant and Grant (1992) noted that “hybridization […] provides favourable conditions for major and rapid evolution to occur.”

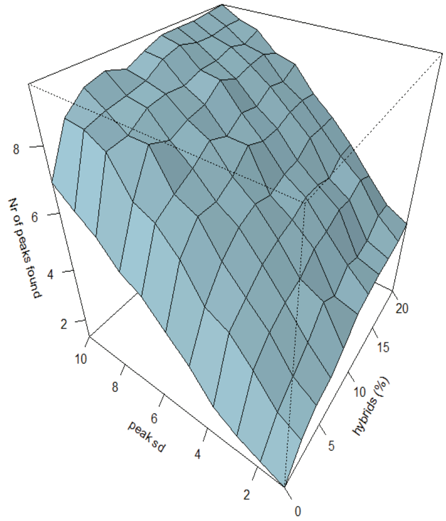


Figure 9.8. The outcome of the model for different combinations of peak width (SD) and percentage of hybrids. The exploration efficiency (measured by the number of peaks found) increases with an increasing percentage of hybrids and with wider peaks. The effect of hybridization on exploration efficiency does however depend heavily on the fitness of the hybrids as determined by postzygotic isolation. In a landscape with wide peaks (i.e. weak postzygotic isolation) more adaptive peaks will be discovered compared to a landscape with narrow peaks (i.e. strong postzygotic isolation).

What do the outcomes of this modelling exercise tell us about the evolutionary history of the True Geese? Did hybridization facilitate the diversification in beak morphology? Several findings from the previous chapters suggest that this might have been the case. First, the model indicates that exploration efficiency is mainly limited by the strength of postzygotic isolation. Species boundaries in the True Geese are preserved by prezygotic isolation mechanisms, only between distantly related species do postzygotic isolation mechanisms come into play (Chapter 6), suggesting high adaptive potential for hybridization between closely related species. Second, the diversification of goose species unfolded during the Pliocene and Pleistocene (Chapter 7), when a circumpolar tundra belt and temperate grasslands emerged (Kahlke, 2014, Prins, 1998), providing numerous ecological opportunities and empty niches. Third, the results from Chapter 8 indicated ancient gene flow among several goose species in this period, suggesting that introgressive hybridization has been an integral component in the evolutionary history of the True Geese.

If and how the evolution of beak morphology in geese was driven by hybridization remains to be investigated. The plausibility of this suggestion is supported by Darwin's Finches (2014, Grant and Grant, 2011): reconstruction of the evolutionary history of the ALX1 gene, which encodes a transcription factor that affects craniofacial development and is strongly associated with beak shape diversity, showed that "natural selection and introgression affecting this locus have contributed to the diversification in beak shapes among Darwin's Finches" (Lamichhaney et al., 2015). I expect that similar dynamics might have shaped the evolutionary history of the True Geese. If so, the True Geese can be regarded as the Darwin's Finches of the Northern Hemisphere.

9.5 Concluding Remarks

The genome is a mosaic of different gene histories, which can be used to study different aspects of the evolutionary history of a species group. In this thesis, for example, I have shown how genomic analyses can provide important insights into the role of hybridization in evolution. The widespread occurrence of introgressive hybridization and horizontal gene transfer across the tree of life should be studied within the Syngameon Scenario, which I introduced in this chapter. Indeed, the large branches on the tree of life can be seen as the slow disintegration of numerous syngameons by selection and drift, of which the resulting lineages diversify and give rise to new syngameons, in an ever expanding network. In this thesis, I showed how one of these branches, the True Geese, flourished about 2 million years ago and is currently disintegrating into independently evolving branches. During this period, hybridization has resulted in the exchange of genetic material between several goose species, possibly driving adaptation to novel environments.

The pervasiveness of hybridization in evolution does however question the tree of life metaphor: "This tree-of-life notion of evolution attained near-iconic status in the mid-20th century with the modern neo-Darwinian synthesis in biology. But over the past 15 years, new discoveries have led many evolutionary biologists to conclude that the concept is seriously misleading and, in the case of some evolutionary developments, just plain wrong. Evolution, they say, is better seen as a tangled web" (Arnold and Larson, 2004). Indeed, trying the capture the complexity of evolution in a branching tree can now be regarded as a wild goose chase.

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Summary

SUMMARY

Hybridization, the interbreeding of different species, has intrigued many evolutionary biologists and has been studied from many different perspectives for several reasons. First, hybridization can be used to gain more insights into the process of speciation, the origin of new species. Second, the exchange of genetic material by means of hybridization (i.e. introgression) can provide the raw material for adaptive evolution. Third, hybridization has become a relevant topic in conservation because it can directly or indirectly contribute to species extinction. In this thesis, I explore the role of hybridization in avian evolution with an emphasis on the so-called "True Geese" (Order Anseriformes, Family Anatidae, Tribe Anserini) using the newest genomic tools. The main body of this thesis consists of two parts, each comprised of three chapters. In Part I (**Chapter 3** to **5**) I deal with hybridization in birds, while in Part II (**Chapter 6** to **8**) I focus on hybridization in the True Geese. In **Chapter 1**, I establish the general framework for this thesis, whereas **Chapter 2** is a stand-alone, introductory chapter clarifying the different concepts that will used throughout the thesis.

In **Chapter 2**, I first consider the "Species Problem" from philosophical and biological perspectives, showing that in order to understand what a species is, one needs to understand what processes and mechanisms underlie the origin and the preservation of a species. In other words, one needs to study the processes of speciation and hybridization. Therefore, I give a short introduction into the several speciation models and the variety of reproductive isolation mechanisms that shape the speciation continuum. Finally, I discuss the historical views on hybridization and highlight some of the creative outcomes of hybridization, namely adaptive introgression, hybrid speciation and transgressive segregation (i.e. extreme hybrid phenotypes).

In **Chapter 3,** I introduce the Avian Hybrids Project, a "peer-reviewed" website gathering the scientific literature on avian hybridization. In addition, I quantify the incidence of hybridization across the avian tree of life: 1714 out of 10,446 bird species (16%) have been documented to have hybridized with at least one other bird species in nature. Including hybridization in captivity, this figure increases to 2204 species (21%).

Chapter 4 is an extensive review on avian hybrid zones and patterns of introgression. I identify 114 avian hybrid zones that have been described from a morphological or genetic perspective. Most of these hybrid zones are classified as tension zones, assuming a balance between dispersal of parental taxa into the zone and decreased fitness of the hybrids. The majority of hybrid zones are probably the outcome of secondary contact after a phase of geographic isolation (i.e. allopatry), but discriminating between primary (i.e. without an allopatric phase) and secondary contact zones is challenging. New techniques, such as Approximate Bayesian Computation (ABC) modelling and patterns of genomic divergence, may be fruitful approaches to tackle this challenge. In addition, several hybrid zones are of recent origin due to anthropogenic disturbances, such as habitat modification and introduction of non-native species. Next, I discuss several striking introgression patterns. First, differential introgression among several genomic classes, such as autosomal, mitochondrial and sex-linked loci, can be explained by Haldane's Rule (which states that if in a hybrid one sex is not viable or sterile, this sex is the one with two different sex chromosomes) and sex-biased dispersal. Second, asymmetric introgression (gene flow primarily from one species into the other) can be the result of a numerous processes, ranging from simple demographic processes, such as range expansion, to complex behaviours, including interspecific forced copulations and brood amalgamation.

The widespread occurrence of introgressive hybridization in birds in combination with other evolutionary processes, such as incomplete lineage sorting and recombination, challenges the use of the classic phylogenetic tree. In **Chapter 5**, I advocate the application of phylogenetic networks to display and analyse complex evolutionary histories. I illustrate the usefulness of these networks by means of two recent examples: the reconstruction of the avian tree of life using phylogenomic techniques and a genomic analysis of the Darwin's finches radiation. The genomic era may result in a paradigm shift in avian phylogenetics, from trees to bushes.

With the general knowledge on avian hybridization from Chapters 3 to 5 in mind, I then turn to the True Geese. In **Chapter 6**, I assemble the available information on hybrid geese by focusing on three main themes: (1) incidence and frequency, (2) behavioural mechanisms leading to hybridization, and (3) hybrid fertility. Hybridization in geese is common on a species-level, but rare on a per-individual level. An overview of the different behavioural mechanisms indicates that forced extra-pair copulations and interspecific nest parasitism can both lead to hybridization. Other sources of hybrids include hybridization in captivity and vagrant geese, which may both lead to a scarcity of conspecifics. The different mechanisms are not mutually exclusive and it is currently not possible to discriminate between the different mechanisms without quantitative data. Most hybrid geese are fertile; only in crosses between distantly related species do female hybrids become sterile. This fertility pattern, which is in line with Haldane's Rule (as females have two different sex chromosomes in birds), may facilitate interspecific gene flow between closely related species.

In **Chapter 7**, I provide a genomic perspective on the evolutionary history of the True Geese. Using an exon-based (i.e. proteincoding DNA sequences) phylogenomic approach, I unravel the phylogenetic relationships among the different goose species. Identical topologies are obtained using either a concatenation or a coalescent-based consensus method. Two major lineages, corresponding to the genera *Anser* and *Branta*, are strongly supported. Within the *Branta* lineage, the White-cheeked Geese (Hawaiian Goose, Canada Goose, Cackling Goose and Barnacle Goose) form a well-supported sub-lineage that is sister to the Red-breasted Goose. In addition, two main clades of *Anser* species can be identified, the White Geese and the Grey Geese. The results from the consensus method suggest that the diversification of the genus *Anser* has been heavily influenced by rapid speciation and by hybridization, which may explain the failure of previous studies to resolve the phylogenetic relationships within this genus. The majority of speciation events took place in the late Pliocene and early Pleistocene (between 4 and 2 million years ago), conceivably driven by a global cooling trend that led to the establishment of a circumpolar tundra belt and the emergence of temperate grasslands, the ideal habitats for geese.

I further investigate the suggestion from Chapter 7, namely that hybridization has influenced the evolution of the True Geese, in **Chapter 8** by unravelling hybridization patterns during and after the diversification of this bird group. Using genome-wide data, I find evidence for ancient interspecific gene flow during the diversification of these goose species. While these high levels of gene flow hinder the precise reconstruction of hybridization events in the *Anser*-clade, it is possible to pinpoint several putative hybridization events within the *Branta*-clade. Hybridization network analyses provide evidence for gene flow between the Red-breasted Goose and the ancestor of the White-cheeked Geese, between Red-breasted Goose and Brent Goose, and between Canada Goose and Cackling Goose. Moreover, the reconstruction of historical effective populations size for all goose species, using the pairwise sequentially Markovian coalescent (PSMC) approach, indicates that most species showed a steady increase during the Pliocene and Pleistocene followed by population subdivision during the Last Glacial Maximum about 110,000 to 12,000 years ago. The combination of large effective populations sizes and occasional range shifts might have facilitated contact between the diverging goose species, resulting in the establishment of numerous hybrids zones and consequent gene flow.

Finally, in **Chapter 9**, I put the results from the previous chapters in broader perspective. First, I introduce the "Syngameon Scenario", a framework to study the evolutionary history of naturally hybridizing species groups. Next, I use this framework to assess how hybridization influences adaptive evolution. The results from a conceptual mathematical model indicate that hybridization can have a positive effect on the exploration of an adaptive landscape. The findings in this thesis show that hybridization is a common and integral component in the evolution and diversification of geese, and birds in general.

Samenvatting

SAMENVATTING

Hybridisatie, het kruisen van verschillende soorten, heeft reeds generaties evolutionaire biologen gefascineerd om een aantal redenen. Ten eerste omdat de studie van hybridisatie inzichten kan opleveren over het ontstaan van nieuwe soorten. Ten tweede omdat de uitwisseling van genetisch materiaal door middel van hybridisatie (i.e. introgressie) genetische variatie kan genereren voor adaptieve evolutie. En ten derde is hybridisatie een relevant onderwerp geworden in natuurbescherming omdat het direct of indirect kan bijdragen aan het uitsterven van bepaalde soorten. In deze dissertatie verken ik de rol van hybridisatie in de evolutie van vogels met nadruk op de "Echte Ganzen" (Orde Anseriformes, Familie Anatidae, Tribus Anserini) aan de hand van de nieuwste genoomtechnieken. Het werk bestaat uit twee grote delen, elk opgesplitst in drie hoofdstukken. In Deel I (**Hoofdstuk 3** tot **5**) behandel ik hybridisatie bij vogels in het algemeen en in Deel II (**Hoofdstuk 6** tot **8**) focus ik op hybridisatie bij ganzen. In **Hoofdstuk 1** schets ik het algemene kader van deze dissertatie, terwijl **Hoofdstuk 2** een opzichzelfstaand introducerend hoofdstuk is waarin ik de verschillende concepten die in deze dissertatie gebruikt worden zal toelichten.

In **Hoofdstuk 2** benader ik het "Soort probleem" vanuit filosofisch en biologisch perspectief om aan te tonen dat om te begrijpen wat een soort is, men moet begrijpen welke processen en mechanismen de oorsprong en de instandhouding van soorten bepalen. Met andere woorden, men moet inzicht verwerven in soortvorming (i.e. speciatie) en hybridisatie. Daarom geef ik een korte introductie over de diverse speciatiemodellen en de verscheidenheid aan reproductieve isolatiemechanismen die het speciatie-continuüm vormgeven. Daarnaast bespreek ik de historische opvattingen rond hybridisatie en benadruk ik enkele creatieve uitkomsten van hybridisatie, zoals adaptieve introgressie, hybride soortvorming en transgressieve segregatie (i.e. extreme hybride fenotypes).

In **Hoofdstuk 3** introduceer ik het Avian Hybrids Project, een "peer-reviewed" website die het merendeel aan wetenschappelijke literatuur rond hybridisatie bij vogels verzamelt. Verder bereken ik het voorkomen van hybridisatie op soortniveau over de volledige stamboom van de vogels: 1714 van de 10.446 vogelsoorten (16%) heeft gekruist met tenminste één andere vogelsoort. Dit getal stijgt tot 2204 (21%) als ik hybridisatie in gevangenschap ook meeneem.

Hoofdstuk 4 is een uitgebreide review over kruisingszones en patronen van introgressie bij vogels. Ik vind ondersteuning voor 114 kruisingszones die beschreven zijn vanuit morfologisch of genetisch perspectief. Het merendeel van deze kruisingszones is geclassificeerd als "spanningszones" (Engels: tension zones), ervan uitgaand dat er een balans is tussen een beweging van oudersoorten naar de zone toe en een lagere overlevingskans voor kruisingen in de zone. Het merendeel van deze kruisingszones is waarschijnlijk het resultaat van secundair contact na een fase van geografische isolatie (i.e. allopatrie), maar onderscheid maken tussen primaire (i.e. zonder allopatrische fase) en secundaire kruisingszones is moeilijk. Nieuwe technieken, zoals Approximate Bayesian Computation (ABC) modellen of patronen van genetische divergentie in het genoom, zijn veelbelovend om deze uitdaging aan te gaan. Verder zijn diverse kruisingszones van recente oorsprong als gevolg van menselijke verstoring, zoals habitatmodificatie en de introductie van exotische soorten.

Vervolgens bespreek ik enkele opvallende introgressie-patronen. Ten eerste kunnen uiteenlopende introgressie-patronen van verschillende genetische klassen, zoals autosomale, mitochondriale en geslachtsgenen, verklaard worden door de Regel van Haldane (i.e. als er in een kruising een van de geslachten niet levensvatbaar of onvruchtbaar is, dan is dit het geslacht met twee verschillende geslachtschromosomen) en geslachtspecifieke verspreiding. Ten tweede kan asymmetrische introgressie (genenoverdracht van één soort naar een andere) het resultaat zijn van diverse processen, van simpele demografische processen als de uitbreiding van het verspreidingsgebied tot complex gedrag, zoals gedwongen copulatie of nestparasitisme tussen soorten.

Het wijdverbreide voorkomen van hybridisatie en introgressie bij vogels in combinatie met andere evolutionaire processen, zoals voorouderlijk polymorfisme en recombinatie, zorgen er voor dat het gebruik van de klassieke fylogenetische boom ter discussie kan worden gesteld. In **Hoofdstuk 5** pleit ik voor de toepassing van fylogenetische netwerken om complexe evolutionaire geschiedenissen weer te geven en te bestuderen. Ik illustreer het nut van deze netwerken aan de hand van twee voorbeelden: de reconstructie van de fylogenetische stamboom van de vogels met behulp van genoomtechnieken en een recente analyse van de radiatie van Darwinvinken. Het genoomtijdperk zal mogelijk leiden tot een verschuiving in fylogenetische analyses bij vogels, van bomen naar struiken.

Met de kennis van Hoofstukken 3 tot 5 in het achterhoofd, vestig ik dan de aandacht op de Echte Ganzen. In **Hoofdstuk 6** verzamel ik alle beschikbare informatie over kruisende ganzen in drie thema's: (1) voorkomen en frequentie, (2) gedrag dat kan leiden tot kruisingen, en (3) vruchtbaarheid van kruisingen. Kruisingen bij ganzen zijn algemeen op soortniveau, maar zeldzaam op populatieniveau. Een overzicht van verschillende mechanismen toont aan dat gedwongen copulatie en nestparasitisme kunnen leiden tot kruisingen. Andere mogelijke oorzaken van kruisingen zijn dwaalgasten en kruisingen in gevangenschap, die beide het gevolg zijn van een gebrek aan soortgenoten. Het is vaak moeilijk om zonder goede kwantitatieve gegevens uit te maken welk mechanisme tot hybridisatie heeft geleid. De meeste ganzenkruisingen zijn vruchtbaar, alleen bij kruisingen tussen niet nauw verwante soorten zijn vrouwelijke kruisingen onvruchtbaar. Dit vruchtbaarheidspatroon, dat in overeenstemming is met de Regel van Haldane (bij vogels hebben de vrouwtjes twee verschillende geslachtschromosomen), kan genetische uitwisseling tussen nauw verwante soorten vergemakkelijken.

In **Hoofdstuk 7** bekijk ik de evolutionaire geschiedenis van de Echte Ganzen vanuit het perspectief van het genoom. Op basis van exonen (i.e. eiwitcoderende DNA-sequenties) bepaal ik de fylogenetische relaties tussen de verschillende ganzensoorten. Twee methoden zijn gebruikt, een concatenatie- (i.e. alle genen worden achter elkaar gelegd en als één groot gen geanalyseerd) en een consensus- (i.e. alle genen worden apart geanalyseerd en er wordt een consensus berekend van de resultaten) methode, beide resulterend in dezelfde relaties. Twee afstammingslijnen, die overeenkomen met de genera *Anser* en *Branta*, worden sterk ondersteund. Binnen de afstammingslijn van *Branta* vormen de witwangganzen (Hawaii gans, Brandgans, Grote en Kleine Canadese Gans) een sterk ondersteunde groep die nauw verwant is aan de Roodhalsgans. De afstammingslijn van *Anser* bestaat uit twee grote groepen: de witte ganzen en de grijze ganzen. De resultaten van de consensusmethode suggereren dat de diversificatie van het genus *Anser* sterk beïnvloed is door snelle soortvorming en door hybridisatie. Dit kan verklaren waarom vorige studies er niet in slaagden de fylogenetische relaties in dit genus te bepalen. Het merendeel van de soortvorming vond plaats tijdens het late Plioceen en het vroege Pleistoceen (tussen 4 en 2 miljoen jaar geleden), waarschijnlijk gestuurd door een globale afkoeling die aanleiding gaf tot het ontstaan van een circumpolair toendrahabitat en gematigde graslanden, de ideale leefgebieden voor ganzen.

De suggestie uit Hoofstuk 7, namelijk dat hybridisatie de evolutie van de Echte Ganzen beïnvloed heeft, wordt verder onderzocht in **Hoofdstuk 8** door de kruisingspatronen tijdens en na de diversificatie van de ganzen in kaart te brengen. Op basis van genoomwijde gegevens vind ik bewijs voor historische introgressie tijdens de diversificatie van deze groep vogels. De hoge mate van genetische uitwisseling bemoeilijkt de reconstructie van mogelijke kruisingsgebeurtenissen in het genus *Anser*. Het is daarentegen wel mogelijk om enkele vermoedelijke kruisingsgebeurtenissen in het genus *Branta* te reconstrueren. Een hybridisatie-netwerk-analyse suggereert dat er genetische uitwisseling heeft plaatsgevonden tussen Roodhalsgans en de voorouders van de witwangganzen, tussen Roodhalsgans en Rotgans, en tussen Grote en Kleine Canadese Gans. Daarnaast geeft een schatting van de historische effectieve populatiegroottes voor alle ganzensoorten, op basis van een "pairwise sequentially Markovian coalescent (PSMC)" analyse, aan dat de meeste soorten tijdens het Plioceen en het Pleistoceen gestaag toenamen, gevolgd door een onderverdeling in subpopulaties tijdens het laatste glaciale maximum ongeveer 110.000 tot 12.000 jaar geleden. De combinatie van deze grote effectieve populaties en af en toe voorkomende veranderingen in verspreiding hebben mogelijk het contact tussen de divergerende ganzensoorten vergemakkelijkt, resulterend in diverse kruisingszones en de uitwisseling van genetisch materiaal.

Tenslotte, in **Hoofdstuk 9**, plaats ik de resultaten van de voorgaande hoofdstukken in een breder perspectief. Eerst introduceer ik het "Syngameon Scenario", een kader waarin de evolutionaire geschiedenis van kruisende soortengroepen bestudeerd kan worden. Vervolgens gebruik ik dit kader om te beoordelen hoe hybridisatie adaptieve evolutie kan beïnvloeden. De resultaten van een conceptueel wiskundig model tonen aan dat hybridisatie een positief effect kan hebben op de verkenning van adaptieve landschappen. De bevindingen van deze dissertatie laten zien dat hybridisatie een algemeen en integraal onderdeel is van de evolutie en diversificatie van ganzen en vogels in het algemeen.

Acknowledgements

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The title of this dissertation – Crossing Species Boundaries – does not only refer to the flow of genes between different promiscuous goose species. It also relates to my own experiences during the course of my PhD. I have crossed many boundaries between countries, chair groups and activities. Furthermore, I have been forced to cross my own boundaries. I would like to thank several people that I have met during my boundary-crossing adventures.

In May 2012, I crossed the boundary between Belgium and the Netherlands to start my PhD in Wageningen. On the first day, I stepped into the office of Herbert Prins. 'So, what should I do now?', I asked shyly. 'Well', said Herbert. 'You have about six months to write a research proposal. You are completely free to choose the topic as long as it is about geese and genetics.' I looked out the window (Herbert resides in the office with the best view) and replied: 'Perfect! I will start tomorrow.' With a friendly slap on my back Herbert replies: 'You lazy dog!' This short conversation was my first close encounter with Herbert Prins: Dutch directness with a loving undertone. Herbert has been very influential during my PhD. Not only regarding the contents of my thesis, but also on a personal level. We have had many enlightening discussions. Especially when we travelled to Salekhard together. The warm personality of Herbert let me forget about the Siberian cold. So, Herbert, thank you for everything! You really made me into a scientist and a better person. 'That's what I think!'

My second promotor is Ron Ydenberg, a Canadian-Dutch (or is it the other way around?) ecologist. A couple of times a year Ron would travel to Wageningen. I always looked forward to these visits. Partly because of the fun parties at Herbert's place, but mostly because we share the same dry humour. The cartoons of Gary Larson always bring a smile to our faces ('Suddenly, Professor Liebowitz realizes he has come to the seminar without his duck.'). The meetings with Ron mostly started with the same phrase. 'So, Jente, it looks like you are doing a great job. How can I help you?' Well, Ron, you helped me by just being there. I really enjoyed spending time with you during coffee breaks, lunches and conferences. You bring a certain joy to the group and to my PhD. Thank you!

There is no such thing as a short meeting with Hendrik-Jan Megens, my co-promotor. Every time I stepped into his office or we met for a coffee a variety of subjects were discussed, ranging from thesis-related problems to personal issues. He also introduced me to the exciting world of bioinformatics. I managed to cross the boundary between biology and informatics, with the help of Hendrik-Jan. So, thank you for everything!

At the Resource Ecology Group, I was guided by two daily supervisors: Sip van Wieren and Pim van Hooft. Their advice has been invaluable during the course of my PhD. Sip, unfortunately we were unable to conduct the crossing experiments we had in mind. I decided to go full-out for the genomic approach. Although, genetic analyses were often a black box for you (and for me, to be honest), your ecological perspective on the problems greatly contributed to my work. And Pim, thé geneticist at REG, we had many lively conversations. Not only with regard to my thesis, but also during coffee breaks where you always provided your own unique perspective to diverse subjects. Pim and Sip, thank you!

REG is also home to two very important ladies that are crucial for the functioning of the group. Gerda and Patricia, thank you for all the administrative hassle. Without you, I would not have been able to travel to conferences, attend courses and – most importantly – finish my thesis.

During my 4+ years working in Lumen and Radix, I have met countless interesting people, distributed over various chair groups. It would take too much time and space to thank them all individually. And if I try to list everyone, I will most probably forget someone (or misspell names). Therefore, a big thank you to all people at REG, ABG, FEM, and NCP. To make sure, you (yes you!) are acknowledged in my thesis, please fill in your name below:

Thank you, ………………………………………..

However, there is one very special person at FEM that I would like to thank personally. Kathelyn, thank you for the support and all the time we spend together in the Netherlands, Belgium, France, Germany, Bolivia and Brazil. I hope we can continue to travel the world together and create more precious memories. Muchas gracias por todo y te amo!

Doing a genetic analysis is difficult without data. And to gather this data, I needed blood samples of all goose species. Several people have been pivotal in the collection of these samples: Gerard Müskens, Jurre Brenders, Henk en Wim Meinen, Ouwehands Zoo (Gerard Meijer), Avifauna (Jan Harteman & Joost Lammers) and the Nederlands Instituut voor Ecologie (NIOO-KNAW). Thank you all. This work would not have been possible without your help.

I have also crossed boundaries on a small scale. My first weeks in the Netherlands, I lived in Ede. Then I moved to Wageningen. Finally, I ended up in Renkum. During my stay in Wageningen, I rented a room at the Hinkeloordseweg. There, I met Dominique and Ton. Thank you for your the great times and good luck with the B&B.

Writing a thesis is a process that involves a lot sitting. Luckily, I could get my much needed movement at WaHo, the student volleyball club. Many thanks to the trainers: Jacques, Flip and Ivo. And of course, a big thank you to all the players that had to deal with my setting and all other members of WaHo for the unforgettable moments in the canteen (with a special thank you for John who supplied us with plenty of beers). Again, to avoid missing anyone in these acknowledgements, please fill in your name below:

Thank you, ………………………………………..

I also crossed the scientific boundary. Many scientists work in their ivory towers and do not often share their research with the public. They rely on science journalists or the communication department of the university. I felt the urge to open the door of my tower and engage with the general public. First, I did this by writing short articles for the Dutch popular science website Scientias. I am grateful to the owners of this website, Caroline and Tim Kraaijvanger, for the opportunity. After finishing my PhD, I started an internship at De Volkskrant. Although they are not directly involved in my PhD, I would to thank the science department of this great newspaper: Tonie, Maarten, Martijn, Cor, Bard, Peter, Ellen, Ianthe, and Anouk.

While communicating science, I crossed another boundary. I went from science into arts and participated in the theatre show ScienceBattle, in which four PhDs try to convince the audience of their research. Many thanks to the people behind this project: Suzanne Streefland and Rene M. Broeders. And also a big thank you to all the participants that I shared the stage with. Your 'kutvragen' definitely helped me prepare for my PhD defence.

Finally, I would like to thank my family who have supported me during my PhD. Dank u, mama, papa, oma, opa (from heaven), ma, pa, Stien en Rob. And of course, thank you to the pets (Lobke, Tisza, Daisy and Mirra) that always welcomed me enthusiastically every time I travelled to Belgium for the weekend.

Curriculum vitae

ABOUT THE AUTHOR

Jente Ottenburghs was born on the 7th of May 1988 in Hasselt, Belgium. He obtained his high school diploma at the Virga Jessecollege Hasselt in 2006, after which he started his Bachelor Biology at the University of Hasselt. In his bachelor thesis, he quantified the genetic diversity of the free-living flatworm *Gyratrix hermaphroditus* in Donana, Spain. This project was supervised by Bart Tessens and Tom Artois. In 2009, he started a Master in Evolution and Behavioural Biology at the University of Antwerp. His Msc thesis focused on the phylogenetic relationships between snail species of the genus *Plutonia* on the Azores, Portugal. Supervisors were Kurt Jordaens and Thierry Backeljau. Because he was enrolled in the module Research, another Msc project needed to be completed. Giving his interest in birds, he studied the singing behaviour of great tits in relation to traffic noise. This research was conducted under the supervision of Héctor Fabio Rivera-Gutiérrez and Marcel Eens. After completing his Msc studies in 2011, he started teaching chemistry and physics at the Katholieke Hogeschool Limburg (KHLim) in Hasselt. In May 2012, he moved to Wageningen to start a PhD at the Resource Ecology Group under the supervision of Herbert Prins and Ron Ydenberg. He studied the genomic consequences of hybridization between different goose species.

Publications

PUBLICATIONS

Ottenburghs, J., van Hooft, P., van Wieren, S.E., Ydenberg, R.C. & Prins, H.H.T. (2016). Birds in a Bush: Towards an Avian Phylogenetic Network. The Auk. 133:577-582 doi: http://dx.doi.org/10.1642/AUK-16-53.1

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PE&RC training and education statement

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (4.5 ECTS)

- The role of hybridization in evolution

Writing of project proposal (1.5 ECTS)

- Hybridization in geese: maintenance of species boundaries despite introgression (2012)

Post-graduate courses (3 ECTS)

- Charles Darwin: history and legacy; Canbridge University (2014)
- Evolutionary biology in Guarda; ETH Zürich and Basel (2015)

Invited review of (unpublished) journal manuscript (2 ECTS)

- Ornis Svecica: hybrids between Snow Goose and Bar-headed Goose (2014)
- Ardea: an expanding hybrid zone between Black-headed and Red-headed Buntings in northern Iran (2016)

Deficiency, refresh, brush-up courses (3 ECTS)

- Advanced bioinformatics course (2013)

Competence strengthening / skills courses (2.5 ECTS)

- PhD Competence assessment; WGS (2013)
- Techniques for writing and presenting a scientific paper; WGS (2013)
- Communication with the media and the general public; WGS (2013)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.8 ECTS)

- PE&RC Day (2012-2015)
- PE&RC Mid-term PhD weekend (2014)

Discussion groups / local seminars / other scientific meetings (8.7 ECTS)

- Ecological theory and application (2012-2015)
- WEES Seminars (2012-2016)
- Netherlands Annual Ecology Meeting; Lunteren, the Netherlands (2013)
- Genetics of Adaptation conference; Uppsala, Sweden (2013)
- European Society for Evolutionary Biology; Lisbon, Portugal (2013)
- The changing world of the goose; Wageningen, the Netherlands (2014)

International symposia, workshops and conferences (9.9 ECTS)

- Goose Specialist meeting; oral presentation; Arcachon, France (2013)
- Genomics of the Speciation Continuum; poster presentation; Fribourg, Switzerland (2014)
- Goose Specialist meeting; oral presentation; Salekhard, Russia (2015)
- International Genomics meeting; oral presentation; San Diego, USA (2016)

Lecturing / supervision of practicals / tutorials (36 ECTS)

- Animal ecology (2012-2014)
- Ecological methods (2013-2015)
- Ecologie (2015)

Supervision of a MSc student

- Possible introgression in geese

COLOPHON

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