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Vocal communication in an avian hybrid zone

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Citation

Hartog, P. M. den. (2008, October 16). *Vocal communication in an avian hybrid zone*. Retrieved from <https://hdl.handle.net/1887/13626>

Version: Corrected Publisher's Version

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Note: To cite this publication please use the final published version (if applicable).

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Introduction, Thesis Overview and Synthesis

Introduction

In this thesis I examine the role of vocalizations in the dynamics of hybridization, or interbreeding, between two species of *Streptopelia* doves: the vinaceous dove, *Streptopelia vinacea*, and the ring-necked dove, *S. capicola*. These two species hybridize even though they have different species-specific signals and are capable of discriminating between these signals. *S. vinacea* and *S. capicola* are not the only species that hybridize. What causes species to hybridize and what are the consequences of this for the dynamics between these species? I explore these issues focusing specifically on the role of vocalizations.

Speciation and reproductive barriers

Speciation, the process by which species are formed, is central to evolutionary biology. If we adhere to the biological species concept, in which species are defined as reproductively isolated populations (Dobzhansky 1937; Mayr 1963), then speciation is the evolution of reproductive isolation. Reproductive isolation can be achieved through mating barriers which, in general, fall into two classes, pre- and postmating barriers. Premating barriers are those that prevent individuals from different species from mating. They include geographic, ecological and behavioural barriers. Postmating barriers include hybrid inviability and infertility, called intrinsic postzygotic isolation, and ecological inviability and behavioural maladaptiveness, called extrinsic postzygotic isolation (Coyne & Orr 2004).

When closely related species that have diverged in geographic isolation, or allopatry, come into secondary contact, reproductive isolation is put to the test. If they have diverged long enough, there may be reproductive barriers in place and they will not interbreed. If they have not diverged enough they may interbreed and ultimately even merge back into one species. However, there is a wide range of possibilities between limited divergence and complete speciation. Determining whether premating barriers exist is done by examining sympatry following secondary contact. To examine whether postmating barriers have evolved, hybridization will provide the necessary evidence. The general assumption is that hybridization does not occur because individuals that breed heterospecifically are expected to incur negative fitness consequences. However, this is not always the case.

Hybridization

Hybridization can have a big impact on the process of speciation. Even though premating barriers may exist and individuals are capable of differentiating con- and heterospecifics, interbreeding between species may still occur. Through hybridization, the genetic divergence between species can be undone altogether (Grant & Grant 2006). On the other hand, if hybrids suffer from reduced fitness, premating barriers may be reinforced and speciation completed. Under certain conditions, hybridization may even give rise to new lineages (Arnold 1997) or may remain a constant phenomenon between two species, without necessarily compromising species boundaries (Gee 2004).

Many taxa sporadically breed heterospecifically in nature and hybridization may occur

due to a variety of factors. Species may not have diverged enough when they come into contact and therefore mate with each other. When coming into contact, one species may be more abundant than the other. This makes it difficult for individuals of the rare species to find conspecific mates, known as Hubbs principle, and facilitates mating heterospecifically as these individuals make “the best of a bad job” (Hubbs 1955; Grant & Grant 1997b; Randler 2006). Under certain conditions, it may even be advantageous to pair heterospecifically depending on the ecological conditions, which may fluctuate within or between breeding seasons (Grant & Grant 1998; Veen *et al.* 2001).

Once hybridization occurs, postmating barriers play a role in preventing further gene flow and bringing the interbreeding to a halt. Postmating barriers are manifested in hybrid fitness. Intrinsic selection against hybrids includes reduced hybrid viability and fertility. Extrinsic selection against hybrids includes ecological and behavioural maladaptiveness. If hybrids are viable, they may have intermediate phenotypes that are not adapted to the environment of either parental species (Kruuk *et al.* 1999). Hybrid mate attraction or territorial signals may be intermediate between the parental species signals and therefore not function adequately in these contexts, effectively leading to behavioural sterility (Coyne & Orr 2004; Gottsberger & Mayer 2007). Hybrid fitness relative to parental species determines the further interactions between the two species. If postmating barriers are in place, that is, hybrids have low fitness, gene flow between the two species will be limited. If postmating barriers do not exist, gene flow may continue and eventually merge the two species into one. Reinforcement, the process by which differences in premating barriers are increased (Dobzhansky 1940; Butlin 1989) depends on the interaction between pre- and postmating barriers. The presence of postmating barriers is a prerequisite for reinforcement of premating barriers.

Areas of secondary contact between species in which hybridization occurs are referred to as hybrid zones. There are several hypotheses that predict the dynamics of a hybrid zone. The bounded hybrid superiority model (Moore 1977; Moore & Buchanan 1985) predicts that the hybrid zone is maintained by selection for hybrids and against parental species within the zone. These hybrid zones are usually found in ecological transition zones, that is, environments to which both parental species are not adapted. The extent of the zone varies with the extent of the ecological transition. The tension zone model states that hybrid zones are maintained by a balance between dispersion of parental species into the zone and selection against hybrids within the zone (Barton & Hewitt 1985). The neutral diffusion hypothesis proposes that in the absence of barriers to gene flow, over time, character clines will become wide relative to dispersal distance (Barton & Gale 1993). Which of these scenarios is occurring will affect the future outcome of hybridization.

Acoustic signals and hybridization

In many species that come into contact, premating barriers suffice to prevent interbreeding. Various signals that function in intra- and intersexual interactions within a species can act as premating barriers, including visual (ultraviolet patterning in butterflies: Silberglied & Taylor 1978;

wing patterns in butterflies: Wiernasz & Kingsolver 1992), chemical pheromones (butterflies: Silberglied & Taylor 1978; Grula *et al.* 1980; European corn borer: Roelofs *et al.* 1987) and acoustic signals. Acoustic signals, in particular those involved in male-male interactions and mate attraction, play an important role in the dynamics between closely related sympatric species. There are examples across a wide variety of taxa of closely related species where acoustic signals prevent interbreeding (lacewings: Wells & Henry 1992; frogs: Hoskin *et al.* 2005; wood crickets: Jang & Gerhardt 2006b). There are also examples in which acoustic signals are different but there is hybridization nonetheless. Natural hybridization in the field between acoustically distinct taxa has been found in field crickets (Doherty & Storz 1992), grasshoppers (Bridle & Butlin 2002; Vedenina & von Helversen 2003), ground crickets (Mousseau & Howard 1998), frogs (Littlejohn & Roberts 1975; Littlejohn 1976; Gerhardt *et al.* 1980) and birds (Alatalo *et al.* 1990; Grant & Grant 1996; de Kort *et al.* 2002a; Secondi *et al.* 2003a; Gee 2005). The structure of vocal signals of two species that have come into contact is crucial in determining the outcome of this contact.

Speciation and hybridization in birds

Birds are an interesting group to study not only because they are very diverse, can be found all over the world, have fascinating plumage and behaviour and can sing beautiful songs. Birds are different from other animals in several ways that affect how they speciate. They can fly and therefore have the ability to colonize remote areas, possibly encouraging speciation (Price 2008). Genetically they are different from other taxa in that females are the heterogametic sex (Price 2008). Male hybrids can be found in more distantly related bird species than in mammals (Fitzpatrick 2004) in part as a result of their double Z chromosome which are both turned on in every cell. Mammals, unlike birds, have a double female XX chromosome, one of which is turned off in each cell making them more susceptible to genetic incompatibilities. Last, but not least, birds differ greatly from other taxa behaviourally. Learning to recognize conspecific characters or learning to produce species-specific signals and sexual imprinting are the main causes of pre-mating isolation in birds (Price 2008). Moreover, speciation in birds usually occurs through behavioural pre-mating barriers to gene flow, with post-mating isolation evolving later (Grant & Grant 1997a). These factors make birds especially interesting for studying the process of speciation and hybridization.

Birdsong functions in territorial defence and mate attraction (Catchpole & Slater 1995; Collins 2004) and is therefore important in species recognition. There are three taxonomic groups within the class Aves that show vocal learning. These are the songbirds (order Passeriformes, suborder Passeri), parrots (order Psittaciformes) and hummingbirds (order Apodiformes, family Trochilidae). Whether birds learn their species-specific song or not can have a great impact on the process of speciation. If song is learned, there is a role for cultural inheritance in the process of speciation. Theoretical studies have shown that learned recognition of conspecifics can accelerate the rate of speciation (Irwin & Price 1999; Lachlan & Servedio 2004; Verzijden *et al.* 2005) and lead to song divergence between populations that have not (yet) diverged genetically (Ellers & Slabbekoorn 2003). Divergence in song then results in parallel divergence in recognition through

imprinting, creating a basis for premating isolation (Price 2008). Studies have shown divergent vocalizations function as reproductive barriers in secondary contact (Baker & Baker 1990; Irwin *et al.* 2001). However, song learning can also prevent or undo divergence when diverged populations learn from one another in sympatry, possibly leading to interbreeding (Grant & Grant 1996; Qvarnström *et al.* 2006). Therefore, song learning can both promote or reverse speciation.

In species that do not learn their songs signal divergence can be expected to reflect genetic divergence more closely. However, their signal recognition mechanism and their responses to species-specific signals may still be acquired through learning or an imprinting-like process (Price 2008). Even though signal acquisition is different in vocal learners and non-learners the responses to these signals may be learned in both groups. This makes non-learners a suitable model for understanding the role of vocal signals in the dynamics of hybridization and speciation.

Around 9% of bird species hybridize regularly in nature (Grant & Grant 1992; Price 2008). Hybridization can greatly affect the structure of vocalizations, which in turn, may affect subsequent hybridization. As these signals are important for a hybrid's reproductive success, their structure is an important component of individual fitness. In many species, hybridization leads to intermediate acoustic signals (Hamer *et al.* 1994) or a wide range of signals ranging from one parental species to the other (Collins & Goldsmith 1998; Ceugniet *et al.* 1999; Deregnacourt *et al.* 2001; de Kort *et al.* 2002a; Gee 2005). The effect of hybridization on these signals depends largely on their inheritance mechanism and whether learning is involved or not. One of the factors that determines the outcome of initial hybridization is how signal structure is affected by hybridization and backcrossing and how hybrid signals are perceived compared to those of the parental species.

What, then, is the role of vocal signals in hybridization? Are the differences between species reinforced? What signals do hybrids have? Do these signals function adequately? And what mediates the response to vocalizations? These are questions I address in this thesis, using a *Streptopelia* dove hybrid zone as a model system.

The study species: Streptopelia doves

Streptopelia doves belong to the order of Columbiformes and are non-songbirds. They develop their species-specific vocalizations without learning from conspecifics. There are seventeen species in this genus (Johnson *et al.* 2001) and they occur Africa, Asia and Europe. They are common where they occur (Goodwin 1983), which makes them good study subjects and many of the species in this genus occur sympatrically. They are similar in morphology and plumage. *Capicola* is slightly bigger than *vinacea* and has more bluish-grey colouring on the head and chest than *vinacea*. *Vinacea* is more pink on head and chest. They also differ slightly in the pattern of black and white on the outermost tail feathers (del Hoyo *et al.* 1997). The most discriminating character between them is their species-specific vocalization, or perch coo (Slabbekoorn *et al.* 1999; Figures 3.1, 4.1 and 5.1). Their vocalizations have a structure that is very suitable for quantitative analyses and comparisons. They are therefore a good model system to study the dynamics of contact between

closely related species that clearly differ in vocalizations.

Male and female doves in the genus *Streptopelia* form monogamous pair bonds and males defend territories in which breeding takes place. Males compete for the acquisition of territories and defend them from other males. They advertise their presence in their territory by uttering the species-specific vocalization, the perch coo, at different conspicuous positions within the territory (Goodwin 1983; Baptista 1996; Slabbekoorn & ten Cate 1996; ten Cate *et al.* 2002). Perch coos are produced in long series called bouts that may consist of three to sixty or more coos. In most species, when an intruder enters an individual's territory, the territory holder will fly towards him, uttering calls while in flight and eventually chase him out of his territory. If the intruder and territory holder land close to each other, they may perform an aggressive display with its accompanying vocalization (bow coo) to the intruder. After having chased away an intruder, the territory owner will usually perch coo.

My research was carried out against a background of research on vocalizations and vocal perception on *Streptopelia* doves (Slabbekoorn 1998; Ballintijn 1999; de Kort 2002; Beckers 2003). This experience with recording, playback experiments and behavioural analyses on *Streptopelia* species served as a guide in understanding the role of vocalizations and designing experiments within these species. Moreover, the experimental setup was validated in previous research. *Streptopelia* species are tuned to their species-specific vocalizations, as shown for the collared dove, *S. decaocto* (Slabbekoorn & ten Cate 1998b). Variation in certain vocal characteristics elicited different responses in *S. decaocto* (Slabbekoorn & ten Cate 1997). Vocal variation between *Streptopelia* species was found to be most distinctive in temporal characteristics (Slabbekoorn *et al.* 1999). This character was also found to be one of the parameters the doves themselves used to discriminate between con- and heterospecific vocalizations (Beckers *et al.* 2003). The evolution of this species-specificity of vocalizations may partly be driven by interspecific interactions (de Kort & ten Cate 2001). However, collared dove males have also been found to respond to a character found in its sister species, the African collared dove (*S. roseogrisea*), but not present in the collared dove (Secondi *et al.* 2003b). In the context of hybridization and intermediate vocal characters, this suggests intermediate hybrid vocal characters may not be responded to by parental species individuals and that this may depend on the specific temporal and frequency structure of these vocalizations. With respect to hybrids, it makes it interesting to study how tuned they are to various vocal characters.

The contact zone in Uganda

The vinaceous dove, *Streptopelia vinacea*, and the ring-necked dove, *S. capicola* (referred to as *vinacea* and *capicola* throughout this thesis), are sister species and have a 2.5% mtDNA divergence (Johnson *et al.* 2001). Their species-specific perch coos are markedly different and the most discriminating character in the field. The two species meet in a narrow contact zone in Uganda (de Kort *et al.* 2002a, b; Chapter 3; Figure 3.2). Birds in the contact zone have intermediate perch coos that range from *vinacea* to *capicola* (de Kort *et al.* 2002a). This suggests hybridization occurs between these species as they do not learn their vocalizations.

The contact zone is found along Lake Albert between the villages of Biiso and Butiaba and is approximately 6 km wide from North to South (de Kort *et al.* 2002a; Figure 3.2). It probably stretches farther North-East from here. I studied adjacent allopatric populations of *capicola* in Queen Elizabeth National Park (N01°46' E31°23'), approximately 270 km south of the contact zone, and *vinacea* in Murchison Falls National Park, south of the village of Paraa and the Victoria Nile (N02°14' E31°34') and approximately 50 km north of the contact zone. These sites were chosen based on the natural distribution of the species. The species are abundant at these sites.

There seem to be no obvious ecological differences between *vinacea* and *capicola*. In general, they occupy a similar habitat and seem to have a similar diet (Urban *et al.* 1986). Both species eat seeds and invertebrates, whether there are differences in the species consumed in the allopatric populations is not clear. Both species are found in dry woodlands, bushy grasslands and open tree savannahs (Urban *et al.* 1986) and the habitat in the hybrid zone is comparable. The *vinacea* population is connected by suitable habitat to the hybrid population. However, the hybrid population is disconnected from the *capicola* population due to patches of unsuitable habitat, like rainforest and agricultural land.

Thesis outline and summary of chapters

In this thesis I elucidate the consequences of hybridization on vocalizations and what impact this has on the dynamics of hybridization between these two dove species. I investigated the patterns of gene flow of the hybrid zone, the response to hybrid vocalizations, the mechanisms affecting responses to vocalizations and the vocal characteristics of F1 laboratory hybrids and the hybrid zone in the field.

The first thing I set out to do was to establish whether the contact zone found in Uganda is a hybrid zone between *vinacea* and *capicola*. In **chapter 2, Directional hybridization and introgression in an avian contact zone: evidence from genetic markers, morphology and comparisons with lab-raised F1 hybrids**, I used molecular techniques to characterize the contact zone and if it is a hybrid zone, understand what type of hybrid zone it is (bounded hybrid superiority, unimodal, bimodal, tension zone). With nuclear AFLP markers, mitochondrial DNA markers, morphological characters and plumage characteristics I analyzed the composition of the contact zone and compared this to the allopatric populations of *vinacea* and *capicola* and F1 hybrid individuals bred in the laboratory. I also kept track of eggs laid and hatched to get preliminary results of F1 viability. F1 hybrids seem to be viable, but comparisons with breeding success and viability of offspring within parental species are necessary to conclude this with certainty. I found that the contact zone is indeed a hybrid zone (Figures 2.1 and 2.2). Both F1 hybrids and hybrid zone individuals were intermediate in AFLP to the parental species. The AFLP markers were not diagnostic enough to distinguish between F1 hybrids and further generation hybrids. The hybrid zone is characterized by high proportion of hybrids and few parental species individuals. This is typical of a hybrid swarm, or a unimodal hybrid zone. On average the nuclear DNA profile, morphology and plumage of the hybrid zone was more *vinacea*, indicating backcrossing to *vinacea*. Most individuals in the hybrid zone and some *vinacea* individuals had *capicola* mitochondrial DNA

haplotypes (Figure 2.3). This indicates asymmetric introgression into *vinacea*. It also suggests that more *capicola* females are mating with *vinacea* males than the other way around. The most likely scenario to explain this, is that *capicola* individuals dispersed into the *vinacea* population, and being the rare species, females had no choice but to mate with *vinacea* males. This is in accordance with Hubbs principle (Hubbs 1955) and most likely due to the geographic distribution of the two species. The *vinacea* population is much closer to the hybrid zone than the *capicola* population.

In chapter 3, **Hybrid vocalizations are effective within, but not outside, an avian hybrid zone**, I investigate the response to hybrid vocalizations within and outside the hybrid zone. Hybrid vocalizations are intermediate to parental species vocalizations and range from one species to the other (de Kort *et al.* 2002a; Figures 3.1 and 4.1). As such, these vocalizations could be dysfunctional in territorial interactions. By playing back territorial vocalizations of both species and of hybrids in both the hybrid zone and the allopatric populations I compared the responses to these vocalizations. I found that within the hybrid zone, individuals respond equally strong to parental species and hybrid vocalizations (Figure 3.4). In the allopatric populations there was a high response to conspecific, an intermediate response to hybrid and a low response to heterospecific vocalizations. In the allopatric populations, hybrids might struggle to defend a territory, as their signals are less effective there. In the hybrid zone though, intermediate hybrid vocal signals are equally effective in territorial interactions as parental species signals. This means that within the hybrid zone, hybrids are most likely not experiencing a disadvantage with respect to parental species individuals when it comes to acquiring and defending a territory. This may contribute to the stability of the hybrid zone.

The experiment in chapter 3, shows that as a population, hybrids respond equally to the three stimuli. This population response could be composed of individuals responding equally to all three stimuli, or individuals responding most to one of the three stimuli, but differing in which one. When averaged, the response to the three stimuli would be similar. To disentangle this I looked at individual responses to manipulated stimuli in **chapter 4, Male territorial vocalizations and responses are decoupled in an avian hybrid zone**. Behavioural coupling, the covariation of signal and response (Hoy 1974; Butlin & Ritchie 1989), may be critical to whether or not two species in a hybrid zone will merge or further diverge. I examined the relationship between an individual's own vocalization and the vocalizations it was responding to in playback experiments to test if there is behavioural coupling of signal and response in individual males. I played back synthetic *vinacea*, *capicola* and hybrid stimuli to hybrid doves. I used a synthetic hybrid stimulus to ensure that it was exactly intermediate between the parental species vocalizations (Figure 4.2). I found that hybrid individuals responded differently to the three stimuli (Figure 4.4). Some individuals responded most to one stimulus, others most to another. Some individuals responded most to two of the stimuli and less to the third. I did not find evidence that an individual's response is dependent on his own signal (Figure 4.5) and this confirms previous findings (de Kort *et al.* 2002b). One explanation for the variation in response is that learning may be involved in determining an individual's response, as has been shown before for territorial species (Catchpole 1978; Irwin & Price 1999). Learning whom to respond to may contribute to the maintenance and

stability of the hybrid zone.

In **chapter 5, Avian vocal variation in one generation of hybridization: F1 lab-bred hybrids similar to individuals from a natural hybrid zone**, I examined the vocal variation of F1 hybrid doves resulting from heterospecific crossings in the laboratory. I compared this to the vocal variation found in the natural hybrid zone in the field. Hybrid zones illustrate that behavioural barriers are not always impermeable and the structure of hybrid vocalizations may play an important role in determining the dynamics of the ensuing interactions between the two species. I found that F1 hybrid vocalizations are intermediate and range from one parental species to the other (Figures 5.1, 5.2 and 5.3). They are similar in range and variation to vocalizations recorded in the hybrid zone. This suggests that the whole range of possible vocalizations is achieved within one generation of interbreeding and that hybrid individuals from the same type of cross may vary greatly with respect to their vocalizations. Hybrids sounding like one of the parental species may not experience a reduced fitness with respect to their vocalizations and be able to settle in the allopatric populations, setting the stage for further introgression. This suggests that this hybrid zone may not only be stable, as found in previous chapters, but that it may expand.

Synthesis

The results in this thesis suggest the following scenario for the origin of the *Streptopelia* dove hybrid zone. Some *capicola* individuals from the allopatric population, that is now 270km away from the hybrid zone and disconnected from it by unsuitable habitat, dispersed into the range of *vinacea*. As conspecific mates were rare, these individuals mated heterospecifically with *vinacea* enabled by partial similarity in their vocalizations. It was *capicola* females, usually the choosy sex in birds, that in effect had no choice but to mate with *vinacea* males. *Capicola* males may not have been able to settle and defend a territory in the *vinacea* population because of reduced response to their perch coos there. Why *vinacea* males would have mated heterospecifically is not clear, but they might have been less choosy, could not find a conspecific female, had multiple matings of which some were heterospecific, or could not discriminate between females from the two species. The subsequent backcrossing to *vinacea* also occurred because *vinacea* mates are more available than *capicola* ones, because the *vinacea* population is closer and the population in which the hybridization started.

The abundance of hybrids in the hybrid zone, evidence for backcrossing to *vinacea*, effectiveness of their signals within the zone, and the breeding of F1 hybrids in the lab indicate that hybrids may not be suffering a loss of fitness, or only to a limited extent, at least not within the hybrid zone. If hybrids within the zone are not experiencing reduced fitness, this indicates the zone is likely to remain a stable area of hybridization between the two species. There is no evidence for the tension zone model, which suggests hybrids should have reduced fitness. It is hard to differentiate at this point between the neutral diffusion hypothesis and the bounded hybrid superiority model because we do not know whether hybrids are as fit, or fitter, than parental species individuals within the hybrid zone. The bounded hybrid superiority model

suggests hybrids are fitter due to an ecologically intermediate habitat. The hybrid zone does not seem to be an ecotone between different habitat types, suggesting this may be a zone in which hybrids are not more or less fit than parental individuals. This also allows for expansion into the allopatric populations, as hybrids would not experience a loss of fitness there due to a different habitat type. However, intermediate behavioural characters will likely be a disadvantage to hybrids in the allopatric populations, but those with parental-like vocalizations may not be.

Hybridization between *vinacea* and *capicola* leads to intermediate vocalizations ranging from one species to the other (de Kort *et al.* 2002a). In this thesis I found that this is already the case within one generation of interbreeding. Therefore, within one generation individuals sounding like one of the parental species are produced. These individuals will probably not have a hard time acquiring and defending a territory in the allopatric parental population they sound like. This, in combination with the circumstantial evidence that they learn whom to respond to in territorial interactions, suggests some hybrids may be capable of dispersing into the allopatric populations and sets the stage for further and extensive gene flow between the two species.

The dynamics of hybridization can proceed differently depending on whether vocalizations are learned or not. In songbirds, secondary contact can create suitable conditions for heterospecific song learning, which in turn may lead to heterospecific mating (Qvarnström *et al.* 2006). In non-songbirds, this cannot happen. However, if responses to signals are learned, even though signals themselves are not learned, secondary contact may allow learning to respond to heterospecific signals which in turn, may also lead to hybridization. Once hybridization has taken place, in non-songbirds it will lead to intermediate songs that may span the whole range including parental-like vocalizations (Lade & Thorpe 1964; Baptista 1996; Collins & Goldsmith 1998; Ceugniet *et al.* 1999; Deregnaucourt *et al.* 2001; de Kort *et al.* 2002a; Gee 2005). It can also lead to strictly intermediate vocalizations (Hamer *et al.* 1994) or vocalizations sounding like one parental species (Delpont *et al.* 2004). In songbirds, depending on the learning mode, songs can be like one parental species or ‘mixed’. In non-songbirds intermediate vocalizations are always a sign of interbreeding but, in songbirds, ‘mixed’ singers are not necessarily hybrids. Mixed songs have elements of the songs of both parental species, but they are different in nature from ‘intermediate’ songs in non-songbirds. These can be intermediate in various frequency and time characters of the songs, creating elements not present in the parental species songs and also include elements of one or both parental species. If individuals learn from their father when they are young, hybrids will learn the song of the father species (Grant & Grant 1996). If they learn later in life from adults they encounter they may sing the song that is most common in the area (Gelter 1987) or sing mixed songs (Alatalo *et al.* 1990; Secondi *et al.* 2003a). Which song an individual learns will have consequences for its fitness. Hybrid individuals, whether they learn or not, sounding like one of the parental species, will likely backcross to that species, facilitating introgression (Grant & Grant 1996). Individuals singing ‘mixed’ songs (songbirds) may be able to defend a territory and attract mates amongst both parental species, one parental species, or neither. Individuals singing intermediate songs (non-songbirds) may only be able to survive and reproduce amongst other hybrids.

In the well-studied hybridization events in songbirds, like the Darwin's Finches of the Galapagos, Flycatchers of the Palearctic and Buntings in North America, hybrids are not very common and there is not a unimodal hybrid zone. In the Darwin's Finches, hybrid fitness depends largely on the ecological conditions (Grant & Grant 1998). They were unfit relative to parental species before a severe El Niño event that changed the ecological conditions on the Galapagos Islands. After this event hybrids survived better than the parental species due to a change in the composition of plants on which the finches depend for food (Grant & Grant 1998). In the Flycatcher and Bunting contact zones, hybrids seem to suffer a loss of fitness (Alatalo *et al.* 1990; Gelter *et al.* 1992; Baker & Boylan 1999). In these cases the divergence time may have been longer to allow these postmating isolation mechanisms to evolve or selection increased genetic differences. Even so, hybridization continues, because for some individuals of the parental species, under certain conditions there are benefits of heterospecific pairing (Wiley *et al.* 2007). In non-songbird contact zones like ours and the one between California and Gambel's quail (Gee 2003), there are many hybrids and the zones seem to be unimodal. Hybrids do not seem to be suffering from a loss of fitness and reinforcement is not likely to occur. Some non-songbird hybrids are easy to breed in the laboratory as shown in this thesis and for partridges and quails (Lade & Thorpe 1964; Collins & Goldsmith 1998; Ceugniet *et al.* 1999; Deregnaucourt *et al.* 2001), suggesting there is not a loss of genetic compatibility with respect to viability. In non-songbirds a stable, bounded, narrow hybrid zone may remain between the two species not necessarily compromising species boundaries (Gee 2003). These patterns raise the question of whether postmating barriers evolve more quickly in songbirds because their premating barriers are plastic.

To conclude, this thesis explored the role of vocalizations in the hybridization process between two non-songbird species. I show that the vocal characters of individuals in the hybrid zone may not be disadvantageous for these individuals. There may be a large variation in these characters, and therefore fitness, between hybrid individuals. In the absence of postmating barriers, speciation may never be complete as there may be situations in which premating barriers alone do not suffice. Hybrid zones such as these, may not be a transient step on the path to complete speciation, but a permanent phase in which species remain distinct with a zone of hybridization between them.