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Vocal communication in an avian hybrid zone

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Male territorial vocalizations and responses are decoupled in an avian hybrid zone

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A core area of speciation research concerns the co-evolution of species specific signals and the selective sensitivity to such signals. Signals and responses to them should be tuned to each other, to be effective in intraspecific communication. Hybrid zones are ideal to study the presence of such 'behavioural coupling' and the mechanisms governing it, and this has rarely been done. Our study examines acoustic signals of males and their response to them in the context of territorial interactions in a natural hybrid zone between two dove species, *Streptopelia vinacea* and *S. capicola*. Male signals are important in hybrid zone dynamics as they are essential for territory establishment, which is crucial for successful reproduction. We tested whether the response of individual male hybrids is linked to how similar their own signal is to the playback signal. We did not find evidence for behavioural coupling. The combined evidence from the low level of response to hybrid and heterospecific signals outside the hybrid zone and a lack of coupling within the hybrid zone, suggests perceptual learning may explain our results. Learning to respond to locally abundant signals may be the best individual strategy and is likely to contribute to the maintenance of a hybrid zone.

Introduction

Species-specific recognition signals can form a behavioural barrier between closely related species with overlapping or adjacent distributions (Gerhardt 1988; Panhuis *et al.* 2001; Slabbekoorn & Smith 2002; Howard & Blomquist 2005). Recognition among conspecifics strongly depends on a match between signal characteristics and perceptual sensitivity (Brumm & Slabbekoorn 2005; Rodriguez & Cocroft 2006). Consequently, co-evolution of signal and response plays an important role in both the process of speciation and the avoidance of hybridization (Alexander 1962; Butlin & Ritchie 1989; Boake 1991). 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 (de Kort *et al.* 2002a).

Behavioural coupling between recognition signals and the response to those signals has been addressed in several invertebrate taxa (Doherty & Hoy 1985; Ritchie 1992; Wells & Henry 1994). Different genetic mechanisms can cause behavioural coupling: pleiotropic effects, genetic linkage or selection causing non-random associations between unlinked alleles (Butlin & Ritchie 1989; Boake 1991). Many studies have also addressed the match between signal variation and response at the population level (perceptual tuning) in frogs, birds, and lizards (Ryan *et al.* 1992; Slabbekoorn & ten Cate 1998b; Quinn & Hews 2000), but as far as we know, no studies have tested the mechanism of behavioural coupling at the individual level in vertebrate species. Nevertheless, the phenomenon may not be restricted to insects and, for example, shared neuronal pathways between brain nuclei for sound production and the circuitry for auditory perception (Williams & Nottebohm 1985; Doupe & Konishi 1991) also provide considerable potential for behavioural coupling in birds.

Learning to respond to signals may result in covariation between signal and response, and forms an important alternative to the genetic or neurobiological explanations for behavioural coupling. Individuals of many species are known to learn early in life, through sexual imprinting, about which signals they should respond to as adults (Immelmann 1975; Andersson 1994; ten Cate & Vos 1999; Galef & White 2000). In birds, where this has been most intensely studied, there is ample evidence that early sensory learning affects responses to both visual and acoustic signals (Grant & Grant 1997b; Irwin & Price 1999; ten Cate & Vos 1999; Riebel 2003). Learning later in life may also lead to a congruence between signal and response, because experience with con- and heterospecifics can enhance or reduce response strength to signals (Catchpole 1978; Irwin & Price 1999).

Investigations into behavioural coupling typically addressed male signals and female preferences for these signals (Hoy *et al.* 1977; Doherty & Gerhardt 1984; Ritchie 1992; Shaw 2000). This is understandable as hybridization ultimately depends on whether males and females of the different species accept each other as partners. However, in strongly territorial species, a prerequisite for male reproduction is the establishment of a territory (Smith 1988). Female mate choice is often based on her assessment of male-male competition (Mennill *et al.* 2002; Leboucher & Pallot 2004; Kunc *et al.* 2006). Females may select mates only after having witnessed

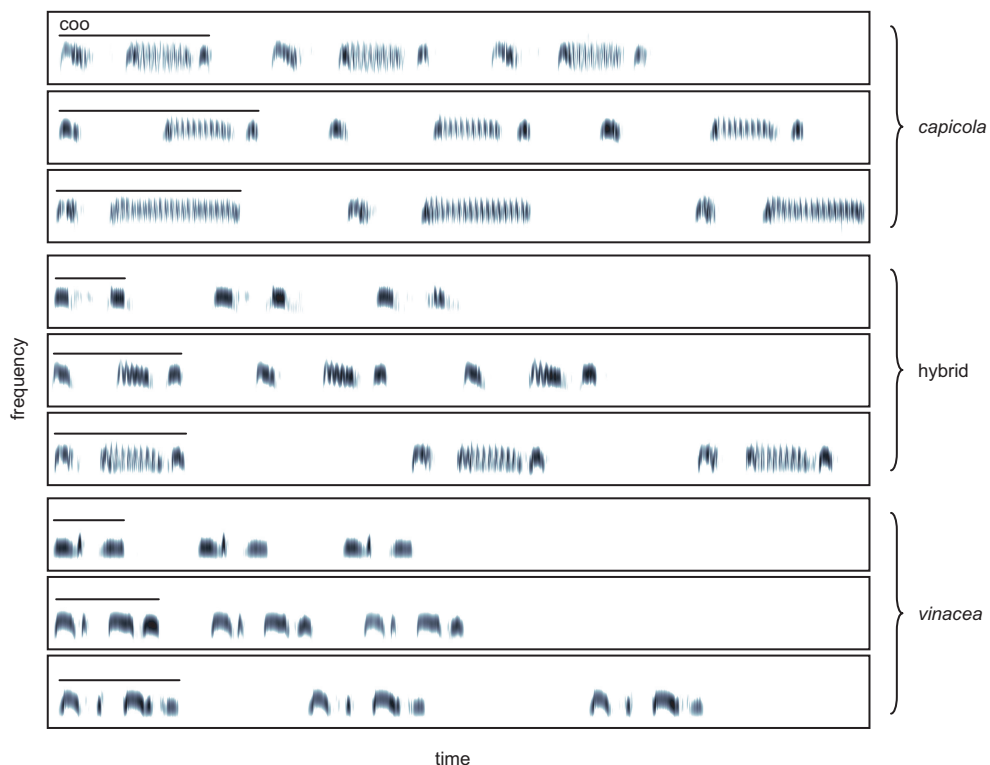


Figure 4.1 Spectrograms illustrating the variation in natural coos of 9 individuals: 3 *capicola*, 3 hybrid, and 3 *vinacea* individuals. Coos were chosen to represent the whole range of natural variation within each group from one extreme to the other. Usually, coos are produced in long series called bouts. Here we show 3 coos per individual of which the first one is marked with a line above it to delineate an individual coo. The time axes are in seconds (0-4) and the frequency axes in Hertz (250-1750). Spectrograms were made using the following settings: Hanning window, sample frequency of 4000 Hz, 400 Fast Fourier Transform samples, window length of 28 samples, overlap of 24 samples and 20 dB dynamic range.

signalling encounters and combat, which have led to resource monopolisation and exclusion of competitors. Consequently, it is important to test whether behavioural coupling plays a role in males. Signals and responses have been shown to correlate within male individuals (Falls *et al.* 1982; McArthur 1986; Moretz & Morris 2003) and may evolve more closely than male signal and female response (Morris & Ryan 1996). It may therefore be more likely to find coupling when it is investigated within the same individuals, instead of between different individuals of different sexes.

Behavioural coupling in territorial males may play a crucial role in the fate of hybrid zones. Hybrid zone dynamics depend heavily on whether hybrids are successfully reproducing within and beyond the hybrid zone. Female avian hybrids often have low viability or fertility (Haldane's rule, Haldane 1922; Coyne 1985), and thus male success may be the critical component. The success of male hybrids will depend on whether territorial signals are recognized and responded to by neighbouring competitors: less response means a less efficient signal and lower chance to establish and maintain a breeding territory (Moore 1987; Price 2008). Hybrid signals may range from those of one to the other parental species (Littlejohn 1976; Mousseau & Howard 1998; de

Kort *et al.* 2002a; Gee 2005) and, therefore, whether or not behavioural coupling is present must have a big impact on the fitness of hybrids across the hybrid zone. Nevertheless, there are no studies which have investigated coupling in an avian hybrid zone.

A great opportunity to study behavioural coupling between signal and response is a recently discovered hybrid zone between two African turtle doves: the Vinaceous dove, *Streptopelia vinacea*, and the Ring-necked dove, *S. capicola* (de Kort *et al.* 2002a; de Kort *et al.* 2002b; from now on referred to as *vinacea* and *capicola*). The vocal variation among *Streptopelia* doves has been well studied (Slabbekoorn *et al.* 1999; ten Cate *et al.* 2002; de Kort & ten Cate 2004). The coo vocalizations are clearly species specific, showing distinct spectral and temporal variation, while the development of the adult signal does not depend on learning (Lade & Thorpe 1964; Nottelbohm & Nottelbohm 1971). Playback experiments in various *Streptopelia* species have shown that territorial males discriminate well between conspecific and heterospecific coo signals (de Kort & ten Cate 2001; Secondi *et al.* 2003b; Chapter 3). *Vinacea* and *capicola* have different, species specific territorial vocalizations and individuals in the hybrid zone have intermediate vocalizations that range from one species to the other (de Kort *et al.* 2002a, b). Furthermore, outside the hybrid zone of *vinacea* and *capicola*, hybrid coos trigger much lower response levels than parental coo types (Chapter 3).

Our previous playback study within the hybrid zone (Chapter 3) revealed equally strong responses to the coos of both parental species and hybrids. This is evidence for the absence of coupling (see also de Kort *et al.* 2002b) at the population level but the large response range could indicate coupling may exist at the individual level. However, in that population-level study we were unable to assess the relationship between playback stimuli and coo characteristics of individual responders. Therefore, we conducted a new series of playbacks in which we recorded both signals and responses of a large set of individuals within the hybrid zone. Again, we played back the typical coos of both parental species as well as a hybrid version to each bird. This time we used digital sound editing to create hybrid vocalizations which were exactly intermediate between the two parental vocalizations. We tested for the presence of behavioural coupling by comparing the individual response strength to the similarity between the birds' own vocalizations and the three stimuli. Presence or absence of behavioural coupling may affect male hybrid fitness with respect to other hybrid and parental phenotypes, which will determine both the nature and the future of the hybrid zone and of the behavioural premating barrier.

Methods

Territorial behaviour in Streptopelia doves

Male doves are territorial and advertise their presence by uttering the species specific vocalization, the perch coo, at different conspicuous positions within their territory (Goodwin 1983; Baptista 1996; Slabbekoorn & ten Cate 1996). Perch coos are produced in long series called bouts that may consist of three to sixty coos. Within a bout, coos are stereotypic (de Kort *et al.* 2002a). 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). After having chased away an intruder, the territory owner will usually perch coo.

The perch coos of *vinacea* and *capicola* are markedly different and hybrids have intermediate perch coos that range from *vinacea* to *capicola* (Figure 4.1 and de Kort *et al.* 2002a). Among other differences, *vinacea* coos usually consist of three to four elements. The first two elements of a *vinacea* coo are sometimes connected and it is hard to separate them. *Capicola* coos usually consist of three elements. Previous playback experiments have shown that both *capicola* and *vinacea* respond more strongly to their conspecific than to the heterospecific and hybrid coos (de Kort *et al.* 2002b; Chapter 3). Hybrids, as a population, respond equally strong to coo types from all three populations (Chapter 3).

Study population

Playback experiments were conducted and recordings were made in a hybrid zone between *S. vinacea* and *S. capicola* in Uganda. The hybrid 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; Chapter 3). AFLP and mitochondrial DNA analyses suggest the hybrid zone is characterised by an abundance of hybrid individuals, backcrossing with *vinacea*, and introgression into *vinacea* (Chapter 2). Individuals in our current study were part of the larger sample taken from the hybrid zone for molecular analyses.

Experimental design and procedure

We carried out 24 trials on 24 different males in the hybrid zone. Three types of synthetic stimuli were presented during each trial, a '*capicola*', a '*vinacea*' and a 'hybrid' stimulus (manipulated stimuli will be written between quotation marks from now on). The three stimuli types were given in all possible orders to allow controlling for order effects. There are 6 possible orders, which were presented 4 times each. Each trial lasted twelve minutes: the pre-playback period consisted of three minutes silence (to measure baseline activities) and was followed by three playback periods each lasting 3 minutes consisting of 1 minute stimulus and 2 minutes of silence. Playback designs such as these, are typically a trade-off between getting a response measure to different stimuli under comparable conditions and avoiding an order effect. The current design was optimized based on experience with previous playback studies on *Streptopelia* doves (Slabbekoorn & ten Cate 1997; de Kort *et al.* 2002b; Secondi *et al.* 2003b; Chapter 3).

Playback trials were carried out from sunrise to 11.00 h and from 16.00 h to sunset between October and November 2004. The experimental protocol, equipment and scoring method are the same as in Chapter 3. Five parameters were scored during a trial: number of coos (coos), number of flights (flights), time spent flying (fly time), number of flight calls (calls) and response latency (latency). The latency was defined as the time between the onset of the playback stimulus and the occurrence of one of the three behaviours described above. In the

pre-playback period, latency was defined as the time from the beginning of the experiment until the occurrence of one of the three behaviours. Each trial was conducted on a different subject and subsequent subjects were at least 200 m apart. Experiments were stopped if the focal male could not be observed or when it interacted with a bird other than his female (see Chapter 3 for more details). This individual was then dropped from the potential subject pool and the stimuli were reused until a successful trial was completed.

Playback stimulus manipulation

We made use of the *vinacea* and *capicola* perch coos recorded in the research area (de Kort *et al.* 2002a), which were also used as unmanipulated playback stimuli in Chapter 3. A total of 72 different stimuli were used: 24 sets of a ‘*vinacea*’, a ‘*capicola*’ and a ‘hybrid’ bout derived from 24 different *vinacea* individuals and 24 different *capicola* individuals to avoid pseudoreplication

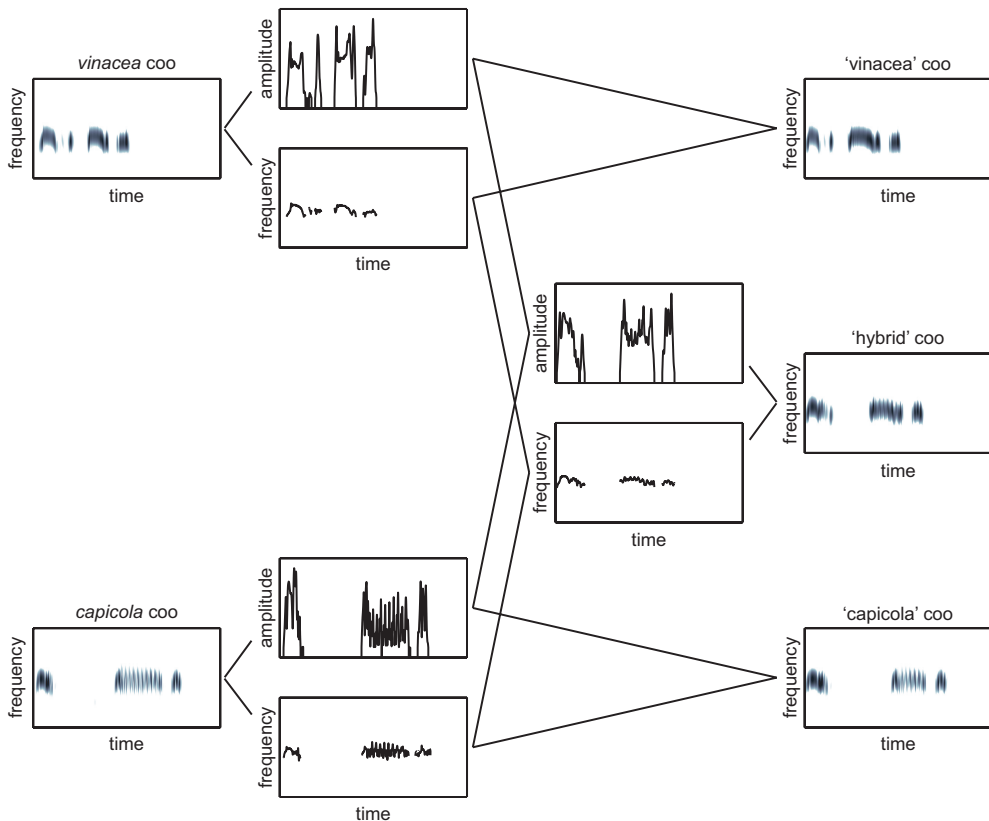


Figure 4.2 Schematic representation of stimulus manipulation. One coo of *capicola* and *vinacea*, here shown in spectrograms, was separated into its amplitude and frequency envelope. These envelopes were then averaged between the two species to create a ‘hybrid’ frequency and amplitude envelope. These ‘hybrid’ envelopes were resynthesized to create a ‘hybrid’ coo. The envelopes of *capicola* and *vinacea* were resynthesized to create manipulated ‘*capicola*’ and ‘*vinacea*’ coos. The time axes are in seconds (0-1.2), the amplitude axes are from 0 to 1, and the frequency axes are in Hertz (0-2000). Spectrogram settings are the same as in Figure 4.1. See *Playback Stimulus Manipulation* in Methods for more details on the manipulation procedure.

(Kroodsma 1989; Kroodsma *et al.* 2001). The *capicola* and *vinacea* recordings used were selected based on the quality of the recording (signal to noise ratio). *Capicola* and *vinacea* coos were paired based on a ‘first come, first serve’ basis, the first coo selected from *vinacea* was paired with the first one from *capicola*. A coo that was not the first, second, or last coo in a *vinacea* and *capicola* bout was selected for manipulation as this is a stereotypic coo in a bout (de Kort *et al.* 2002a). The amplitude and frequency envelope were extracted from each coo. For a pair of one *capicola* and one *vinacea* coo, these envelopes and the duration were averaged per element to create a ‘hybrid’ coo. The amplitude envelope is the maximum amplitude at each point in time. The frequency envelope is composed of the corresponding frequencies of the amplitude envelope plotted in time. The envelopes were derived from spectrograms made using the following settings: Hanning window, sample frequency of 4000 Hz; 400 Fast Fourier Transform samples; window length of 28 samples; overlap 24 samples and 20 dB dynamic range. They were normalized to 90% of the full dynamic range. They were then resampled to the average length between the two elements, which vary in length (Figure 4.2, and for similar manipulation techniques see Secondi *et al.* 2003b; Slabbekoorn & ten Cate 1997; Slabbekoorn & ten Cate 1998b).

These amplitude and frequency envelopes were used to synthesize a manipulated ‘hybrid’ coo. The resampled *capicola* and *vinacea* envelopes were also used to resynthesize a ‘capicola’ and a ‘vinacea’ coo. As a result, ‘hybrid’ and ‘parental’ coos used as stimuli had undergone the same manipulation which avoids differences in response due to the manipulation procedure. Elements in the resynthesized ‘vinacea’ and ‘capicola’ coos were of the average length between the original elements. To create the pauses between the elements in a ‘hybrid’ coo, the length of the pauses in the *capicola* and *vinacea* coos were also averaged. For our manipulation procedure, the first two elements of *vinacea* were considered one element. This yielded three-element coos for both *vinacea* and *capicola* coos and allowed us to take the average of each element.

The manipulated coos were repeated a number of times to create a bout for playback. For the ‘hybrid’ stimulus, they were repeated as many times as the average number of coos in the original *vinacea* and *capicola* bouts. For the ‘vinacea’ and ‘capicola’ bouts they were repeated as many times as the original *vinacea* and *capicola* bouts had coos. To create one minute of sound, bouts were copied as many times as necessary to fill one minute of playback. Bouts were separated by a pause between them lasting 10% of the bout length. If a bout was halfway at the end of a minute, care was taken that the minute ended with a full coo. The stimuli were played back at a sample rate of 44.1 kHz. The amplitude of the playback stimuli ranged from 75-80 dB at one meter from the speaker. We created 24 sets in total, each consisting of a ‘vinacea’, ‘capicola’ and a ‘hybrid’ stimulus. Stimulus manipulation was done in Matlab 7.0.1.

Recordings and difference measures

Recordings were made before, during and after the playback experiments with a Sennheiser ME 67 shotgun microphone with a K6 battery power supply module, a low noise microphone preamplifier (150Hz - 10 kHz frequency response) and a Creative Nomad Jukebox 3 at a sample rate of 44.1 kHz.

We calculated various difference measures between the own coo and the coos played back, to assess whether an individual's response depended on how similar its own coo was to the coos played back to it. This allows an assessment of the relation between the response to each playback stimulus and the similarity of an individual's coo to that same stimulus. For each individual, one to five bouts were chosen based on availability and recording quality. Again, one coo that was not the first, second, or last coo of each bout was selected to be analysed. Each of these coos was compared to the manipulated 'vinacea', 'capicola' and 'hybrid' coo. Two procedures were used to calculate difference measures: one analogous to the manipulation procedure and one based on time and frequency parameters of the coos. All the difference measures were calculated using Matlab 7.0.1.

For the first procedure, three difference values were calculated, one each for the: amplitude envelope, frequency envelope and duration. In the synthesis procedure averages between element envelopes were calculated but, in the difference procedure, envelopes were subtracted from each other to yield a difference envelope. The Root Mean Square (RMS) of these envelopes was then calculated for each element. The average of the RMS difference envelopes for each element was calculated. The smaller the mean value, the more similar the envelopes are. For duration, the sum of all differences in length of elements and pauses was used as a difference measure. The smaller the sum, the more similar the coos are. These differences were then averaged for the five coos measured.

For the second procedure, a set of difference measures was created by measuring specific acoustic parameters from the spectrogram of the subject and stimulus coos. The parameters measured were coo length (from the beginning of the first element to the end of the last element), the average frequencies of element 1 and 2 (the RMS of the frequency envelopes of each element), the loudest frequencies of element 1 and 2 (the frequency in each element that had the highest amplitude). The difference between each coo and a stimulus coo was calculated by subtracting the measures from each other. These differences were then averaged for the five coos and the absolute value was calculated. For the differences in frequency (average frequency and loudest frequencies) a PCA was carried out. The first factor of this PCA (PC1 Frequency Measures) was used as a difference measure of frequency. The two sets of difference measures resulted in a total of five difference measures per individual: each calculated for the 'vinacea', 'capicola' and 'hybrid' stimulus coo. These are the differences in amplitude envelope, frequency envelope, duration, coo length and PC1 Frequency Measures.

To visualize the data and present it in Figure 4.4, individuals were classified into three groups, those vocally more similar to 'capicola', more similar to 'vinacea' or intermediate. This was done by creating one measure based on our five difference measures. For each individual, we subtracted the difference to 'vinacea' from the difference to 'capicola'. Individuals with a value higher than 1 were classified as vocally similar to 'vinacea', individuals with a value lower than -1 were classified as vocally similar to 'capicola' and those with values between -1 and 1 as intermediate.

Table 4.1 Correlation matrix for the response variables included in the PCA. loadings for all variables for the response PC1 (first component) are also shown.

response variable	flights	coos	calls	latency	fly time	loadings response PC1
flights	1.000					0.838
coos	0.143	1.000				0.370
calls	0.778	0.264	1.000			0.954
latency	-0.114	-0.50	-0.166	1.000		-0.260
fly time	0.577	0.230	0.824	-0.161	1.000	0.879

Statistics

A Principal Components Analysis (PCA) was used to create one response variable from the five variables that were scored (coos, flights, fly time, calls, and latency). This is a widely used method for analyzing playback response measures suggested by McGregor (1992). The first factor of the PCA (Response PC1) was used as the response variable in several linear mixed models in SAS 9.1.3 (Proc Glimmix). Two linear mixed models were made in which the variable “stimulus” was entered as a fixed parameter. Repeated measures were taken into consideration by entering individuals as random effects. The first linear mixed model assessed whether the playbacks had an effect on an individual’s territorial behaviour by contrasting the response to each stimulus to the baseline activities in the pre-playback period. In the second linear mixed model, “stimulus” was entered as a fixed effect and the pre-playback period was excluded from the data, to assess whether there were differences in response to each stimulus. Order effects (whether a stimulus was played first, second or third) and order-stimulus interactions were included in the model.

A new set of five models was made to evaluate the relationship between the five difference measures and the response. In these models Response PC1 was again used as the response variable and “stimulus” was removed as a fixed effect because it was not statistically significant. “Order” remained in the models as a fixed effect because it was statistically significant. By leaving “order” in the models, the variation explained by it is accounted for. The difference measures were entered as fixed effects and individuals as random effects.

Results

General response to playback

Individuals responded to the playback stimuli by flying up and searching for the simulated intruder, uttering flight calls and cooing. These measures were combined into one measure of response by a PCA and the first component, Response PC1, was used in further analyses (explained 52% of the variation). The correlation matrix showed strong correlations between flights, fly time and calls and less between these variables and coos and response latency (Table 4.1). Kaiser-Meyer-Olkin measure of sampling adequacy=0.641. Bartlett’s test of sphericity: $\chi^2_{10}=258.20, P<0.001$.

There was a statistically significant increase in response to all three stimuli, ‘vinacea’, ‘capicola’ and ‘hybrid’, compared to the pre-playback baseline activities (pair-wise comparisons of the least-squares means estimates; ‘capicola’: $t_{69} = -4.90$, $P < 0.0001$, estimate(error) = $-0.673(0.137)$; ‘hybrid’: $t_{69} = -3.63$, $P = 0.0005$, estimate(error) = $-0.497(0.137)$; ‘vinacea’: $t_{69} = -3.81$, $P = 0.0003$, estimate(error) = $-0.523(0.137)$; stimulus effect in Linear Mixed Model: $F_{3,69} = 9.09$, $P < 0.001$, Figure 4.3). The overall response at population level did not differ significantly among the three stimuli (Linear Mixed Model, $F_{2,40} = 1.66$, $P = 0.2033$, Figure 4.3). There was an effect of the order ($F_{2,40} = 9.91$, $P = 0.0003$) in which the stimuli were played: the first stimulus elicited a higher response than the second and third stimulus. The stimulus-order interaction ($F_{4,40} = 1.48$, $P = 0.2278$) was not significant.

Response level and signal-stimulus difference parameters

There was considerable variation among individuals with respect to coo characteristics and response strength (Figure 4.4). Variation in response patterns is not related to whether individuals

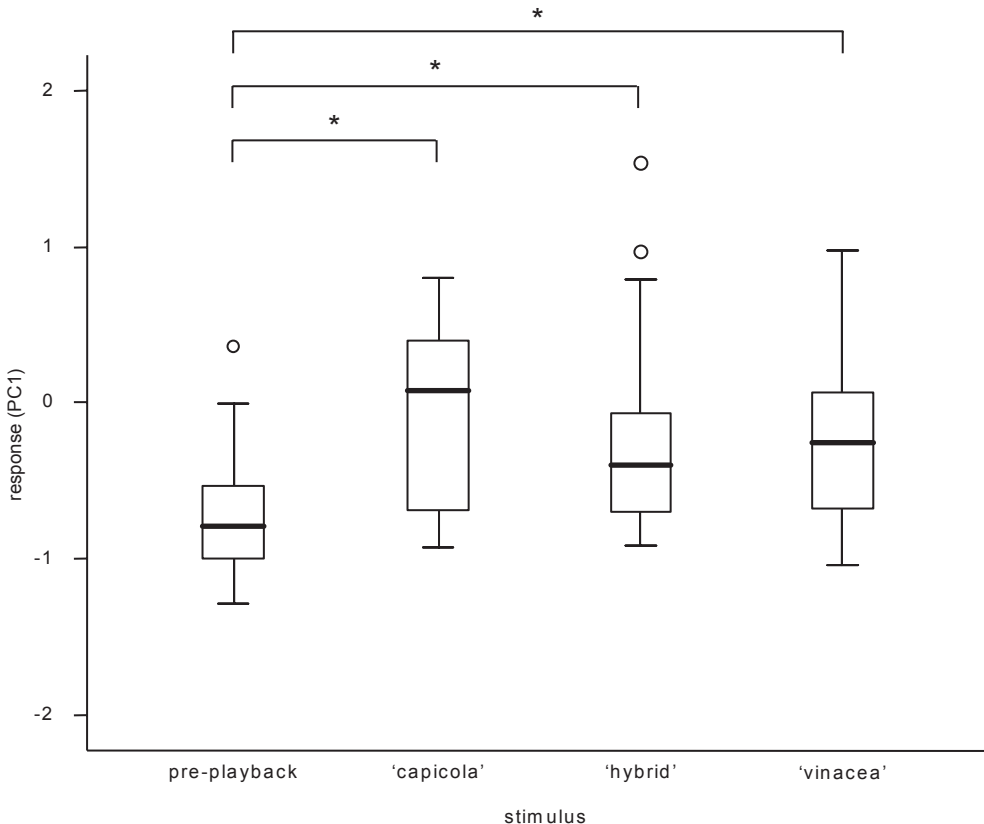


Figure 4.3 Responses to each of the three playback stimuli, ‘capicola’, ‘hybrid’, and ‘vinacea’ significantly increased compared to the pre-playback baseline activities. Asterisks represent significant differences. The Response PC1, is the first factor of the PCA of the five scored response variables. Box plots represent the median, interquartile range and full ranges.

Table 4.2 Effect of measures of differences in amplitude, frequency, duration, coo length and frequency measures (PC1) between an individual's own coo and the stimulus coo (see Methods for the calculation of the difference measures). There is no effect of difference measures on the response to the stimuli. CI=confidence interval, df=degrees of freedom. "Order" is the fixed effect entered into the model to test for effects of the order in which the stimuli were played (see Methods).

difference measure (range) or effect	estimate	standard error	num df	den df	F	P	95% CI	
							lower	upper
amplitude difference (0.1961 - 0.4119) order	-0.8477	1.4014	1	45	0.37	0.5483	-3.6702	1.9749
			2	45	8.34	0.0008		
frequency difference (238 - 447) order	-0.00032	0.001246	1	45	0.07	0.7981	-0.00283	0.002189
			2	45	8.71	0.0006		
duration difference (796 - 6143) order	0.000086	0.000047	1	45	3.37	0.0731	-8.37E-06	0.00018
			2	45	7.45	0.0016		
difference coo length (0 - 0.41) order	0.1534	0.525	1	45	0.09	0.7714	-0.9039	1.2108
			2	45	8.86	0.0006		
PC1 frequency measures (-1.5 - 2.762) order	0.004589	0.05302	1	45	0.01	0.9314	-0.1022	0.1114
			2	45	8.81	0.0006		

are similar to 'vinacea' or 'capicola'. Some respond equally to all three, some respond the most to one of the three, and others respond a lot to two stimuli and not to the third.

We did not find a relation between response and the difference between own signal and playback stimulus when tested in the linear models. Figure 4.5 shows the lack of a relationship between response strength and differences in amplitude envelope and frequency envelope. If response strength to a particular stimulus is related to similarity to that stimulus this would result in a decreasing response with increasing difference, i.e. in a negative relationship. This is not the case. None of the five difference parameters was statistically significant in determining the response strength (see Table 4.2 for linear model results).

PC1 Frequency Measures explained 62% of the variation in the measures of frequency differences. The loadings of these measures were: mean frequency of element 1=0.820 and element 2=0.609; loudest frequency of element 1=0.895 and element 2=0.806. The correlation matrix showed correlations between all the measures. Kaiser-Meyer-Olkin measure of sampling adequacy=0.718. Bartlett's test of sphericity: $\chi^2_6=98.99, P<0.001$.

In this study we were not able to find a relationship between an individual's coo and response characteristics. We have presented estimates (effect sizes), their ranges, their standard errors and confidence intervals in Table 4.2. The confidence intervals include zero indicating that the effects are not statistically significant. The effect sizes were small and often had large standard errors. The effect size and standard error of the variable "duration difference" is such that by increasing the sample size to around 60 individuals it may be possible to find an effect if it is there. However, for the other variables samples sizes would have to range from 500 to 25,000 to find an effect if it is there. In those cases finding a significant effect may not have a biological meaning.

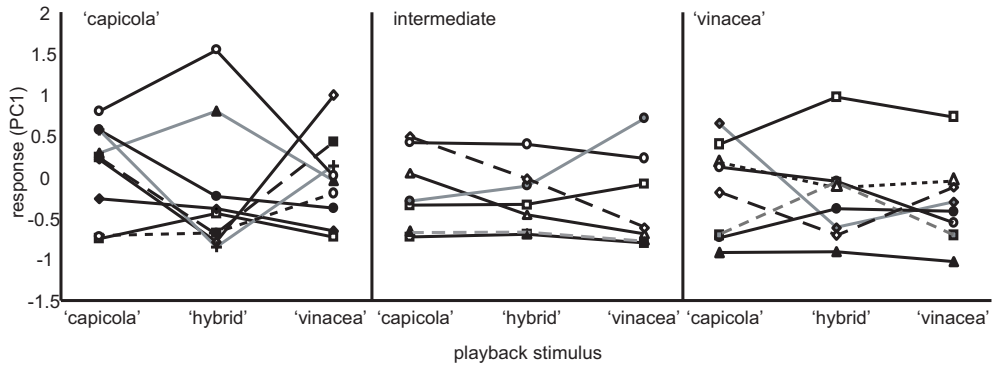


Figure 4.4 Responses of all individuals to the three stimuli. Individuals are divided into three groups: those vocally more similar to 'capicola', more similar to 'vinacea' or intermediate (see Methods). Individuals respond very differently to the three stimuli and this is independent of the similarity of their coo to the stimuli played back to them.

Discussion

Territorial males within the turtle dove hybrid zone responded significantly to all three types of vocalizations: 'vinacea', 'capicola' and intermediate 'hybrid' stimuli in comparison to the pre-playback baseline activities. However, despite homogenous patterns at the population level, response strength was highly variable among and within males. Some responded to all three stimuli and others responded most to one or two of the three stimuli. Furthermore, the response to the stimuli varied independently of the own vocal characteristics of the responding individuals. These results suggest the absence of behavioural coupling between signal and response.

In a previous study, we showed a match between signal variation and perceptual sensitivity at the population level, indicating population divergence in signal and response between parental and hybrid populations (Chapter 3). We now know that the wider acceptance range in response to different stimuli for the hybrid population is not necessarily an individual trait, but the result of large variation in response patterns among individuals. Moreover, we have seen that individuals may respond to all vocalizations equally or they may have a response bias to one particular stimulus. When there is a bias to respond to a particular stimulus, that particular stimulus also varies between individuals. The implications for our understanding of the inheritance of signal and response and the consequences for hybrid zone dynamics will be addressed below.

Signal and response are not coupled

Our findings indicate the lack of behavioural coupling in male doves from a hybrid zone (see also de Kort *et al.* 2002b). We now can rule out some of the potential genetic mechanisms that could link production and perception of territorial signals in general (Hoy *et al.* 1977; Doherty & Gerhardt 1984; Ritchie 1992; Shaw 2000). It is unlikely that pleiotropic effects or close genetic linkage determine covariation in signal and response, as these mechanisms would have persisted in the hybrid zone. On the other hand, we can not rule out genetic linkage between relatively distant loci, as this might be broken down in backcrosses and F2 hybrids, which are likely well-

represented in our data (Chapter 2). Also, signal and response may be governed by independent sets of genes, coupled due to linkage disequilibrium. This coupling would eventually disappear in a hybrid zone, but would take longer in narrow zones with influx from both parental species. Finally, signal and recognition might be controlled by the same or linked genes, but the response may be controlled by other genes that have become decoupled in the hybrids (Roelofs *et al.* 1987).

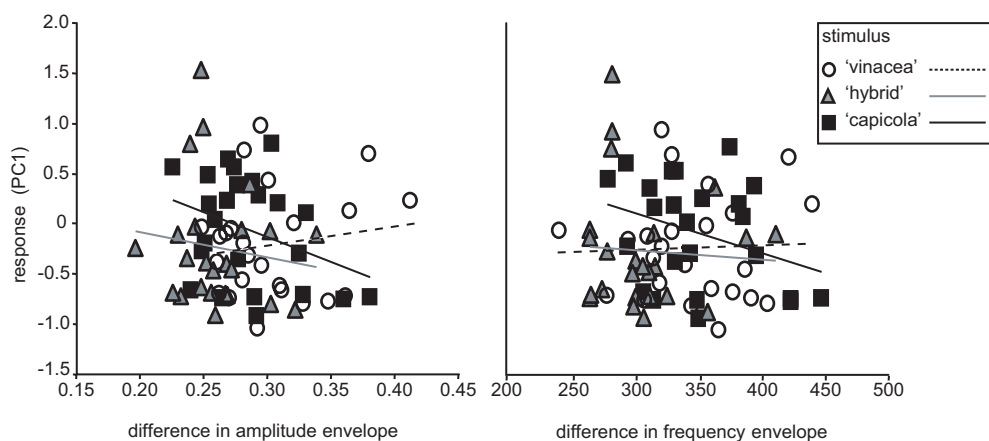


Figure 4.5 Response to the playback stimuli plotted against two measures of difference between an individual's own coo and the stimulus coo played back to it. The Response PC1, is the first factor of the PCA of the five scored response variables. The two difference measures are (a) difference in amplitude envelope, (b) difference frequency envelope (in Hz). Linear regression lines show the relation between response to stimuli and difference between own coo and stimulus coo for each stimulus. The response of an individual to the playback stimuli was independent of how different the stimulus was to an individual's own coo (see Results).

An alternative explanation for the seemingly random association between signal and response at the individual level might be that the response patterns do not relate to any type of genetic predisposition as addressed above, but depend on experience. One possibility is that male response is based on early experience with the father's coo (e.g. Hitchcock *et al.* 1989). This would result in variation with respect to the specific stimuli eliciting the strongest response, which would not necessarily match the own vocalization. However, this cannot account for individuals with a broad response range or with two response peaks to both parental coo types. Another possibility is that response tendencies remain plastic until after dispersal and territory establishment, which allows for adjustment to one or more neighbours through learning when adult (as can also happen in song production learning, McGregor & Krebs 1989; Beecher *et al.* 1994; Nelson 2000; Nicholson *et al.* 2007). In this case, variation in individual response characteristics could relate to the number and features of neighbours which might be learned during territorial interactions. This could account for broad and narrow response ranges and single as well as double-peaked response patterns.

It is well known that songbirds are able to learn to recognize conspecific songs and to which songs to respond to as adults (Riebel 2003). Response control and potential for neuronal

plasticity may be similar for doves and songbirds as they use the same areas of the brain for recognition of species specific vocalizations (Terpstra *et al.* 2005; Terpstra *et al.* 2006). Adult *Streptopelia* doves have also been shown to be capable of learning discrimination tasks in an operant set-up based on conspecific and heterospecific vocalizations (Beckers & ten Cate 2001; Beckers *et al.* 2003). Hence, hybrid males in the current study may have learned to respond to the vocalizations of nearby competitors, similar to the learned heterospecific responses in several cases of closely related bird species with ecological overlap (Catchpole 1978; Irwin & Price 1999; Hansen & Slagsvold 2003; Sedláček *et al.* 2006). If learning is responsible for the observed pattern, this could be either early or adult learning, which would lead to different relationships between a male's response and the coo characteristics of either his father or his neighbours.

Implications for hybridization

The fate of a hybrid zone depends a great deal on whether hybrids are successfully reproducing within and beyond the hybrid zone. Male success may be the key component as female avian hybrids often have low viability or fertility according to Haldane's rule (Haldane 1922; Coyne 1985). Therefore, signal and response characteristics of males are essential to understand whether the hybridizing species will continue to merge or whether the hybrid zone will remain or disappear (Moore 1987; Price 2008). Irrespective of the mechanisms governing signal and response in parental species, in hybrids they are decoupled. We will discuss the fitness consequences for male hybrids within and outside the hybrid zone, taking their vocal and response characteristics into account.

In the allopatric parental populations, male hybrids with intermediate or heterospecific vocal features may have a low settling success rate. The low response levels to hybrid signals in these populations suggest hybrid signals are deficient in territorial communication (Chapter 3). However, male hybrids with vocal features close to one of the parental species may potentially be successful in establishing a territory outside the hybrid zone. Their success will likely vary depending on whether their response to territorial signals is determined by genetic predispositions or based on post-dispersal learning of the local neighbourhood. We expect that the ability to learn to respond to the local signals as adults might make hybrid individuals successful in the allopatric populations. Responding appropriately to local signals would be less readily achieved if responses are dependent on genetic predispositions.

In the hybrid zone, male hybrids seem equally suitable to establish a territory as parental species males. The current study shows homogenous average response levels to the whole range of coo variation from one parental type to the other as has been shown in a previous study (Chapter 3). However, if response characteristics also affect success rate in territory establishment, individuals with broad response ranges would do better and be more abundant in the hybrid zone. It is hard to predict whether responses that depend on genetic predispositions will be adapted to local signals. But, flexible response characteristics and adult males that have the capacity to learn about the hybrid vocal variation among neighbours, would lead to responses adapted to local signals. Response ranges and biases would then be concordant with, and dependent on, vocal

features of neighbours. Response ranges might be broad as neighbours can vary a great deal in the hybrid zone but they might also be narrow depending on the particular vocal characters of an individual's neighbours. If learning plays a role in responding, this provides plasticity by which hybrids might do well within the hybrid zone irrespective of their own coo type, while hybrids with parental-like coo types might be able to establish themselves in parental populations. This may contribute to the maintenance of the hybrid zone and introgression into parental species.

Conclusion

We examined the occurrence of behavioural coupling at the individual level in an avian hybrid zone. We demonstrate the lack of coupling between signal and response features and can now exclude pleiotropy and close genetic linkage between male signal and male response in *Streptopelia* doves. We suggest that learning is a likely explanation for the observed response patterns even though we cannot rule out genetic effects on responses. The capacity of post-dispersal adjustment of response irrespective of the own vocal features, in combination with divergent average response patterns within and outside the hybrid zone may contribute to hybrid fitness and ultimately to patterns of introgression. These findings may apply more widely to other avian systems in which learning affects response patterns but does not affect vocal development.

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