Vocal communication in an avian hybrid zone

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Avian vocal variation and hybridization: F1 lab-bred hybrids similar to individuals from a natural hybrid zone

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Manuscript

Species-specific vocalizations often function as a pre-mating barrier between species. Hybrid zones illustrate that such 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. We examined vocal variation resulting from hybridization in the African *Streptopelia vinacea* x *S. capicola* dove natural hybrid zone and compare this with the variation found in F1 hybrids reared in the lab. Such a comparison can reveal whether the large range of variation in acoustic signals often found in hybrid zones can be achieved in one generation of hybridization or results from several generations and allows for an assessment of how genetic background affects vocal structure. Doves are ideal to explore this issue as they do not learn their species-specific vocalizations, which precludes cultural inheritance affects the structure of vocalizations. We found F1 hybrid vocalizations are intermediate and range from one parental species to the other and are similar in range and variation to that found in the natural hybrid zone. The whole range of possible vocalizations is achieved within one generation of interbreeding and hybrid individuals from the same type of cross vary greatly with respect to their vocalizations. Hybrids sounding like one of the parental species may not experience a loss of fitness and be able to settle in the allopatric populations, setting the stage for further introgression. This suggests the hybrid zone will remain stable between the two species and possibly expand.

Introduction

Species-specific 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 many examples of closely related species where acoustic signals prevent interbreeding across a wide variety of taxa (Grant & Grant 1996; Hoskin *et al.* 2005; Jang & Gerhardt 2006). However, there are also examples of species whose acoustic signals are apparently not sufficiently distinct to prevent hybridization (de Kort *et al.* 2002a; Gee 2005). Hybridization can be a crucial step in the process of speciation as it can complete speciation through reinforcement of premating barriers or undo it altogether through the merging of species (Price 2008). One of the factors that will determine the outcome of initial hybridization is how signal structure is affected by hybridization and how hybrid signals compare to those of the parental species.

The characteristics of hybrid signals, relative to parental species signals, will determine their success in interactions with parental species. The effect of hybridization on acoustic signals has been studied in both natural hybrid zones and laboratory crosses in various species. There is a diversity of outcomes depending on the species and context. In some insect species, F1 hybrids bred in the lab have acoustic signals with intermediate characteristics (lacewings: Wells & Henry 1994, 1998; field crickets: Hoy *et al.* 1977, Walker 2000) and there can be a sex-linked effect on these signals (grasshoppers: Vedenina & von Helversen 2003, Gottsberger & Mayer 2007, crickets: Bentley & Hoy 1972; Shaw 1996). F2 hybrid signals have been found to also be intermediate and similar to the F1 hybrid signals (crickets: Shaw 1996, grasshoppers: Vedenina & von Helversen 2003, Gottsberger & Mayer 2007). Backcrossing to the parental species usually results in individuals with signals similar to the species they are backcrossed to (grasshoppers: Saldamando *et al.* 2005a, crickets: Shaw 1996). Hybrids found in natural hybrid zones in the field can be in-between, similar to one species, or different from both in their signals (field crickets: Doherty & Storz 1992). They may also be intermediate and span from one species to the other (grasshoppers: Bridle & Butlin 2002, Vedenina & von Helversen 2003, ground crickets: Mousseau & Howard 1998). Some frog species show the whole range of possible signals between parental species already in the F1 laboratory hybrid generation (Doherty & Gerhardt 1983, 1984) while various types of hybrids in the field can have the typical species-specific signals of one of their parental species (Gerhardt *et al.* 1980; Lode 2001). Hybridization in the field may also lead to intermediate signals varying from one species to the other (Littlejohn & Roberts 1975; Littlejohn 1976). Intermediate signals have also been found in hybrid seals studied under field conditions (Page *et al.* 2001).

In songbirds, natural hybridization can lead to 'mixed' singing, i.e. individuals singing elements of the songs of two species (Alatalo *et al.* 1990; Secondi *et al.* 2003a) and can cause a mismatch between song and phenotype within individuals (Gill & Murray 1972b; Grant & Grant 1997c). However, in these species it is hard to disentangle the effects of learning and genetic admixture as 'mixed' songs may be due to learning and not necessarily interbreeding. Non-songbirds do not learn their signals (Nottebohm & Nottebohm 1971) and the consequences of genetic admixture on their signals are unambiguous. Therefore when examining the effect of hybridization on avian vocalizations, these species are good model systems. Several studies of non-songbird hybrid vocalizations, both F1 lab-bred hybrids (Lade & Thorpe 1964; Baptista 1996; Collins & Goldsmith 1998; Ceugniet *et al.* 1999; Deregnaucourt *et al.* 2001) and natural hybrids in the field (de Kort *et al.* 2002a; Gee 2005) show that they may span the scope of signals between their parental species. Field hybrids have also been found to have signals like one parental species (Delport *et al.* 2004) or be intermediate (Hamer *et al.* 1994).

Variable and altered hybrid signals may affect a hybrid individual's ability to defend a territory and attract mates and thus its fitness. Females of pure parental species may respond to intermediate male hybrid signals as they would to parental signals in some species (Deregnaucourt & Guyomarc'h 2003) but in others they have shown a clear preference for conspecific signals (Bridle *et al.* 2006). Hybrid signals may also attract females of both species (Littlejohn & Watson 1976a). In a competitive context, playbacks of hybrid signals to parental species males have elicited an intermediate level of response in comparison to the response to conspecific and heterospecific signals (Collins & Goldsmith, 1998; Chapter 3) or similar to that to conspecific signals (Ceugniet & Aubin 2001). The variation in hybrid signals probably leads to a large variation in fitness for hybrid individuals and signals sounding like one parental species may facilitate introgression. It is therefore critical to understand the acoustic variation in hybrids to determine the fate of a hybrid zone and potential for introgression between species.

The differences in variability and intermediacy of hybrid signals found in studies of different species groups will partly reflect the differences in genetic composition between laboratory, usually F1 hybrids, and field hybrids which may include several generations of hybrids and backcrossed individuals. Inheritance patterns in different taxa may also explain the differences. Although hybrid vocal signals have been investigated in various contexts, to our knowledge, a direct comparison between known F1 hybrids and natural hybrids in the field has not been done in vertebrates. Such a comparison can reveal whether the large range of variation in acoustic signals often found in hybrid zones can be achieved in one generation of hybridization or is the result of interbreeding over several generations. Our aim is to provide such a study. We examined vocal variation resulting from hybridization in non-songbirds that hybridize naturally in the field: the African *Streptopelia vinacea* x *S. capicola* hybrid zone and compare this with the variation found in F1 hybrids in the lab.

The Vinaceous dove, *S. vinacea*, and the Ring-necked dove, *S. capicola* (from now on referred to as *vinacea* and *capicola*) are sister species (Johnson *et al.* 2001) that are morphologically similar but have distinct species-specific territorial vocalizations (Figure 5.1; de Kort *et al.* 2002a). They hybridize in a narrow contact zone in Uganda (de Kort *et al.* 2002a, b; Chapter 3). Our genetic analyses on the contact zone show an abundance of hybrid individuals and backcrossing with and introgression into *vinacea* (see Chapter 2). However, we were not able to classify specific individuals genetically as F1 or F2 hybrids or backcrosses. The acoustic variation among hybrids in the hybrid zone seems to cover the full range between the two species (de Kort *et al.*, 2002a). We bred F1 hybrid individuals in the lab and compared their vocal characteristics to those of

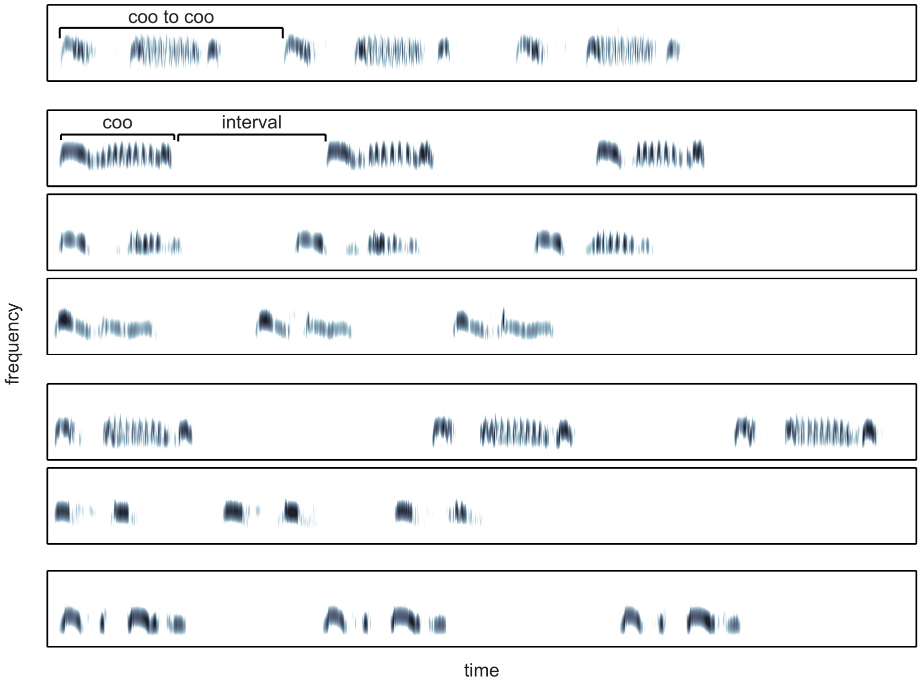


Figure 5.1 (continues on opposite page) Spectrograms illustrating the variation in natural coos of 14 individuals: 2 *capicola*, 6 F1 hybrid, 4 field hybrid, and 2 *vinacea* individuals. Coos were chosen to represent the whole range of natural variation within each group from one extreme to the other. Usually, coos are produced in long series called bouts. Here we show 3 coos per individual. The measures of Coo to coo, Coo and Interval are depicted in two of the spectrograms with lines. 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.

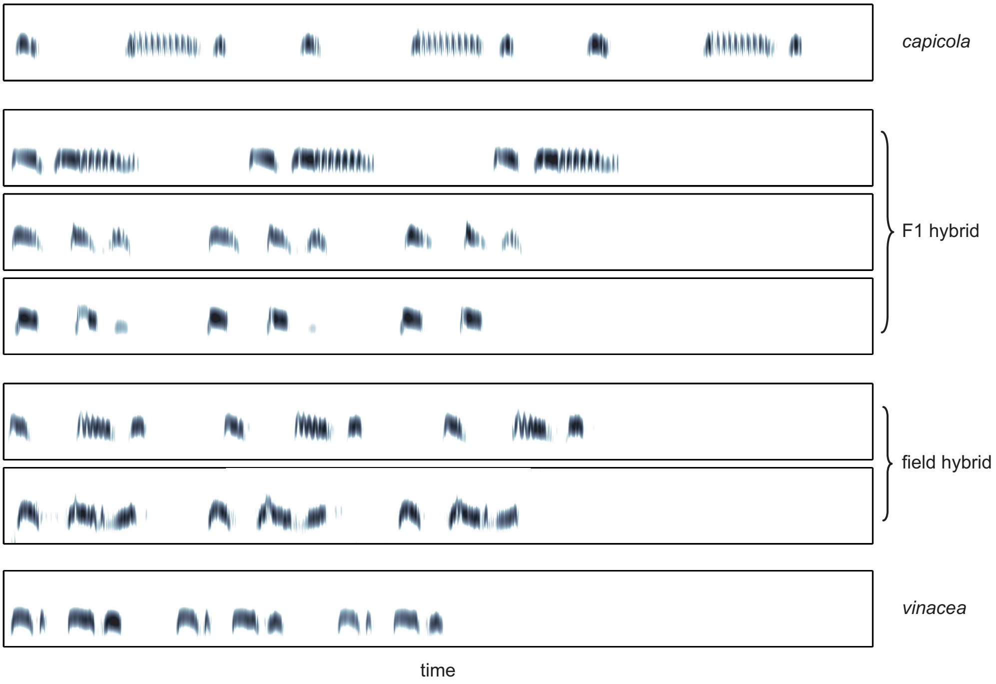
hybrids in the field. We address the following questions: How do the vocal parameters of field hybrids and F1 hybrids compare to those of the parental species? Do F1 hybrids and natural field hybrids differ in vocal characteristics and the variation therein? Are hybrids more variable within or between individuals than parental species and does this differ between F1 hybrids and natural field hybrids? Do F1 hybrids from reciprocal crosses differ in their vocal characteristics? Are F1 brothers vocally more similar to each other than non-brothers?

Methods

Study populations

Vinacea and *capicola* are sister species and have a 2.5% mtDNA divergence (Johnson *et al.* 2001). The species-specific territorial vocalizations, perch coos, are markedly different and the most discriminating character in the field (Figure 5.1). The two species meet in a narrow hybrid zone in Uganda (de Kort *et al.* 2002a, b; Chapter 3).

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). The hybrid zone is characterized by an abundance of hybrid individuals and there is backcrossing with *vinacea*. There is also evidence for introgression into *vinacea* (Chapter 2). We studied adjacent allopatric populations of *capicola* in Queen Elizabeth National Park (N01°46' E31°23'), approximately 270 km south of the hybrid population, 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 hybrid population. These sites were chosen based on the natural distribution of the species. The species are abundant at these sites.

Vocalizations

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; ten Cate *et al.* 2002). Perch coos are produced in long series called bouts that may consist of three to sixty coos. The perch coos of *vinacea* and *capicola* are markedly different and birds in the hybrid zone have intermediate perch coos that range from *vinacea* to *capicola* (de Kort *et al.*, 2002a). Previous playback experiments have shown that both *capicola* and *vinacea* respond more strongly to conspecific coos than to the heterospecific and natural 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).

F1 crosses

In the allopatric populations 19 *vinacea* and 18 *capicola* were captured using mist nets at water holes. These individuals were taken to our laboratory in Leiden. These individuals bred to produce F1 hybrids. Six pairs with a *vinacea* female and a *capicola* male resulted in 10 F1 hybrid males and seven pairs with *capicola* female and *vinacea* male resulted in 13 F1 hybrid males. Birds were sexed using molecular markers described in Chapter 2. Each pair had one to three sons (Table 5.1). Eggs were left to hatch with their natural parents, unless they were abandoned. In this case, eggs were 'cross fostered' to domesticated *Streptopelia risoria* doves (Table 5.1). Recordings of individuals were made when they were between 15 and 36 months old. Birds were reared in the common dove rooms and exposed to adult coos of *vinacea*, *capicola* and other F1 hybrids (cross fostered birds were also exposed to adult coos of their foster parents).

Table 5.1 F1 males recorded in this study, their parents and whether they were cross fostered or not. Parents' names beginning with a "C" are *capicola*, those beginning with a "V" are *vinacea*.

F1 hybrid	father	mother	cross fostered	F1 hybrid	father	mother	cross fostered
250	C10	V9	yes	261	V10	C13	yes
253	C10	V9	yes	258	V12	C14	yes
286	C15	V5	no	259	V12	C14	yes
287	C15	V5	no	260	V12	C14	yes
256	C17	V13	no	282	V14	C21	no
262	C2	V11	yes	289	V14	C21	no
275	C2	V11	no	292	V15	C5	yes
297	C20	V16	yes	252	V4	C8	yes
230	C7	V7	no	263	V4	C8	yes
299	C7	V7	no	290	V8	C12	yes
248	V1	C3	no	291	V8	C12	yes
255	V1	C3	no				

Recordings

Field - We made recordings of 10 *capicola* males: 6 were recorded in the *capicola* population between October and December 2000 by Selvino de Kort (de Kort *et al.* 2002a) and 4 were recorded by PMdH between November and December 2003. Ten *vinacea* males were recorded in the *vinacea* population by Selvino de Kort between October and December 2000 (de Kort *et al.* 2002a). Twenty-one males were recorded in the hybrid zone between September 2003 and January 2004 and between September and November 2004 by PMdH, we refer to them as "field hybrids" from now on. Recordings were made with a Sennheiser ME 67 shotgun microphone with a K6 battery power supply module, a low noise microphone preamplifier (frequency response 150Hz - 10 KHz) and a Creative Nomad Jukebox 3 at a sample rate of 44.1 kHz.

Lab - From the captive population in the laboratory we recorded 23 F1 hybrid males (referred to as "F1 hybrids" from now on), 7 *capicola* males and 12 *vinacea* males. For recording, males were housed individually in a cage (40cm high x 53cm wide x 40cm deep) in a sound-attenuating chamber for 1 to 7 days. A Sennheiser ME67 condenser cardioid microphone was placed at 0.5 to 1 meter from the bird and connected to a K6 module, Sennheiser MZN 16 P48 power supply and a RDL RU-MP2 preamplifier (frequency response 25Hz - 20kHz). Recordings were made on a Creative Nomad Jukebox 3, at a sample rate of 44.1 kHz and 0dB gain. Recordings were made automatically: when sound was detected between 400 and 1100Hz a device would remove the 'pause' from the jukebox , which would start recording.

Spectral analysis of vocalizations

For each individual 5 bouts were analysed. For individuals for which we did not have 5 bouts we used 3 (1 F1, 1 field hybrid, 5 *capicolas* and 2 *vinaceas* recorded in the field) or 4 (4 field hybrids, 3 *capicolas* and 2 *vinaceas* recorded in the field) depending on the amount of recordings available. In each bout, one coo at the beginning, middle and end of the bout was chosen for measurement. The first and last coos of a bout were excluded because they can be very variable or incomplete. From the lab recordings 5 bouts per individual were chosen randomly for the analysis. From the field recordings, bouts were chosen based on recording quality. Bouts had to consist of at least 5 coos to be included in the analysis. The selected bouts were entered into the Luscinia sound analysis program (http://luscinia.sourceforge.net). In this program, spectrograms were made of the vocalizations and measurements of individual elements were made in the spectrogram. The settings for creating the spectrograms were: frame length 15ms, and time step 3ms. Depending on the recording quality the dynamic range varied from 60 to 75 dB and the echo removal from 30 to 75%. These parameters were varied in order to adjust for the inevitable variation in field recordings. Each coo was classified as a 'syllable' and the elements of a coo were marked as 'elements.' Elements were defined on the basis of silent gaps of 20ms or more. However, this was inadequate in many cases partly because of recording quality and partly because elements are sometimes stuck together. In these cases we used our judgement to segment elements.

Luscinia measures contours for various acoustic parameters in an element. Contours are constructed from measurements made at each spectrum in the spectrogram. We used the following parameters in our analyses: time, peak frequency, peak frequency change over time, and trill rate and amplitude. For the trill rate and trill amplitude, "trill" refers to the rapid, often sinusoidal frequency modulation of an otherwise tonal signal. Luscinia carries out a short-term FFT of the peak frequency contour to estimate these parameters. For each possible trill rate, the program draws a sine wave with that frequency and matches it with the data. The trill amplitude is calculated as the amplitude of the sinusoidal oscillations in the peak frequency contour. We carried out two types of analysis of these contours. The first involved extracting summary statistics that in our opinion described much of the acoustic variation between coos, while the second involved using a computation approach to compare elements with each other on the basis of the contours. Assessing variation in complex signals like dove coos is still a problem without a clear solution. By using these two alternative approaches, we used examples from two of the major traditions in the field.

Statistics

We used the following element measures: length, gap length, average peak frequency, average trill rate, and average trill amplitude. Parameters describing the whole coo (overall parameters) were calculated from these measures. Time measures calculated from the element measures were: Coo to Coo (length from the beginning of the first element to the beginning of the first element of the next coo), Coo (length from the beginning of the first element to the end of the last element) and Interval (length of the gap between coos, from the end of the last element to the beginning of the first element of the next coo). Overall Peak Frequency, Overall Trill Amplitude and Overall Trill Rate were calculated by taking the average element measures, multiplying it by the element length, summing these values for all elements in a coo for each parameter, and dividing them by the sum of the element lengths.

A set of six linear mixed models was made in SAS 9.1.3 (Proc Glimmix) to test for differences in the acoustic parameters between populations (Population=*capicola*, *vinacea*, F1 hybrid, field hybrid) and recording locations (Location=field or lab). The variables "Population" and "Location" and an interaction between them were entered as fixed effects. All the bouts measured from an individual were used in the analysis. Repeated measures were taken into consideration by entering individuals as random effects. The acoustic parameters (Coo to Coo, Coo, Interval, Overall Peak Frequency, Overall Trill Amplitude, Overall Trill Rate) were entered as response variables. Some of these parameters did not meet normality assumptions and did not follow alternative distributions that linear mixed models allow for so they were transformed to better follow the Gaussian distribution. The reciprocal (1/x) was taken of Coo to Coo and Interval. The natural log (ln) was taken of Coo and Overall Trill Amplitude. The transformed variables were then used as response variables in the models. Location or the interaction between Population and Location had a significant effect on the time parameters (Coo to Coo, Coo and Interval, Table 5.2) so we did not lump the field and lab recordings of *vinacea* and *capicola*. We made all pair wise comparisons within recording location as Location alone accounted for some of the variation found. Multiple pair wise comparisons were corrected with the Tukey method (Games & Howell 1976).

A new set of six linear mixed models was made to test for the effects of pair composition (Cross type: *vinacea* mother x *capicola* father or *capicola* mother x *vinacea* father), Cross fostering (cross fostered or not) and the interaction between these two. We found no effects of cross fostering on the vocal parameters and therefore removed it and the interaction term with Cross type from the models. All the bouts measured from an individual were used in the analysis. Repeated measures were taken into consideration by entering individuals as random effects.

Another set of six linear mixed models was made to test if brothers were acoustically more similar than unrelated males. To this end, individual fathers (Father) were entered as fixed effects in the model. All the bouts measured from an individual were used in the analysis. Repeated measures were taken into consideration by entering individuals as random effects.

To visualize the variation between individuals and present it in a Figure (Figure 5.3), a Principal Components Analysis (PCA) was used to create two components (PC1 and PC2) from

Table 5.2 Effect of population and location on overall vocal parameters. df, degrees of freedom. Bold values indicate significant *P* values.

parameter	effect	numerator df	denominator df	F	*P*
reciprocal coo to coo	population*location	1	77.60	3.22	0.0764
	location	1	77.60	4.26	**0.0423**
	population	3	77.42	24.67	**<.0001**
ln coo	population*location	1	77.34	6.82	**0.0108**
	location	1	77.34	0.16	0.6944
	population	3	77.13	68.11	**<.0001**
reciprocal interval	population*location	1	77.53	0.11	0.7412
	location	1	77.53	7.43	**0.0079**
	population	3	77.34	7.99	**0.0001**
overall peak frequency	population*location	1	77.20	0.01	0.9034
	location	1	77.20	0.08	0.7759
	population	3	77.14	34.17	**<.0001**
ln overall trill amplitude	population*location	1	77.53	3.06	0.0842
	location	1	77.53	1.39	0.2417
	population	3	77.40	65.57	**<.0001**
overall trill rate	population*location	1	77.50	0.18	0.6759
	location	1	77.50	2.27	0.1361
	population	3	77.32	6.16	**0.0008**
DTW PC1	population*location	1	78.00	0.74	0.3922
	location	1	78.00	0.14	0.7095
	population	3	78.00	124.24	**<.0001**

the six acoustic variables measured: Coo to Coo, Coo, Overall Peak Frequency, Overall Trill Amplitude, and Overall Trill Rate. The first two components explained 82% of the variation in the data. PC1 represented all the parameters except the Overall Trill Rate which had the highest loading in PC2. The correlation coefficients between the parameters ranged from 0.207 to 0.842. The component loadings for the first and second component, respectively were: Coo to Coo (0.818, -0.039), Coo (0.899, -0.209), Overall Peak Frequency (0.793, -0.350), Overall Trill Amplitude (0.931, 0.061) and Overall Trill Rate (0.531, 0.830). The Kaiser-Meyer-Olkin measure of sampling adequacy=0.0763; Bartlett's test of sphericity: $\chi^2_{10}=240.770$, $P<0.001$. Interval was not included in the PCA because it showed a very weak correlation with the other variables.

Dynamic time warping

Three bouts of an individual were compared with each other and to three bouts of all other individuals of the other population. This was done with a Dynamic Time Warping (DTW) algorithm in Luscinia (http://luscinia.sourceforge.net). DTW is a well-established algorithm that aligns time-series (in our case, our acoustic parameter contours) to allow them to be compared. It calculates distance measures for any two given signals. DTW is comparable to spectrogram cross-correlation, but produces more reliable comparisons for signals that are only partially similar. We tried different weightings for the parameters Time, Peak Frequency, Peak Frequency Change, Trill Amplitude, Trill Rate and Gap. Changing the weightings of the parameters had little effect on the comparison results, so we chose the simple option of weighting the parameters equally (all=1, all other weightings were set to equal 0). We set the compression factor to 1.2, SD ratio to 0.9, offset removal to 0, the cost of stitching to 1.2. We carried out a Nonlinear multidimensional scaling (Kruskal 1964) ordination of the results of the DTW analysis and generated 6 components. We used the first of these for further analysis. DTW PC1 was also entered as a response measure in the linear mixed models to test for differences between groups (as above).

Permits

Individuals were caught with permission from the Uganda Wildlife Authority (permit no. 00455) and the Uganda National Council for Science and Technology (permit no. EC 578). Live birds were exported with permission from the Uganda Wildlife Authority (Material Transfer Agreement no. 028, License to export live, non-scheduled animals serial no. 2505), the Uganda National Council for Science and Technology and the CITES Authority (permit no. 001477) in Uganda. Live birds were imported into the Netherlands with permission from the Voedsel en Waren Autoriteit (TRVV/52190). Recordings made of vocalizations of captive animals were approved by the Leiden University committee for animal experiments, license number: DEC05065.

Results

Parental species vocalizations

Population had a significant effect for all parameters tested (Table 5.2). In the pairwise comparisons parental species vocalizations showed statistically significant differences in all parameters measured (except Interval, Table 5.3 and Figure 5.2). Individuals of the two species do not have any overlap at all in vocal characteristics (Figures 5.1, 5.2 and 5.3). *Capicola* and *vinacea* vocalizations are therefore distinct.

F1 hybrid and parental species vocalizations

For the overall vocal parameters, F1 hybrids had a mean value that was intermediate between the means of the two parental species recorded in the lab (except for the parameter Interval, Table 5.4, and Figure 5.2). Their vocal characters span the character space between the two parental species (Figures 5.1, 5.2 and 5.3). F1 hybrids differ significantly from both parental species recorded in the lab in Coo to Coo, Overall Peak Frequency, Overall Trill Amplitude and DTW PC1 (Table 5.3). For Coo they differed only from *vinacea*, and for Interval only from *capicola*. For Overall Trill Rate they did not differ from either parental species. Taken together, this suggests they are not only intermediate, but form a distinct vocal group from both parental species.

The variation between individuals is comparable to that in the parental species as shown

Table 5.3 Pair wise comparisons of vocal parameters per Location. Least-squares mean estimates are shown, df, degrees of freedom. Bold values indicate significant *P* values. *P* values were adjusted for multiple comparisons (see Methods) yielding an Adjusted *P*.

parameter	location	populations compared		estimate	standard error	df	t	*P*	adjusted *P*
reciprocal coo to coo	field	*capicola*	*vinacea*	-0.00042	0.000057	78.75	-7.48	<.0001	**<.0001**
		capicola	hybrid	-0.00016	0.000049	78.80	-3.37	0.0012	**0.0033**
		hybrid	*vinacea*	-0.00026	0.000048	77.66	-5.36	<.0001	**<.0001**
	lab	*capicola*	*vinacea*	-0.00028	0.00006	76.59	-4.61	<.0001	**<.0001**
		capicola	F1	0.000164	0.000054	76.63	3.02	0.0035	**0.0095**
		F1	*vinacea*	-0.00011	0.000045	76.65	-2.50	0.0145	**0.0381**
ln coo	field	*capicola*	*vinacea*	0.5482	0.04608	78.68	11.90	<.0001	**<.0001**
		capicola	hybrid	0.3632	0.03959	78.75	9.17	<.0001	**<.0001**
		hybrid	*vinacea*	0.185	0.03943	77.42	4.69	<.0001	**<.0001**
	lab	*capicola*	*vinacea*	0.3734	0.0486	76.17	7.68	<.0001	**<.0001**
		capicola	F1	-0.3381	0.04412	76.22	-7.66	<.0001	**<.0001**
		F1	*vinacea*	0.03521	0.0364	76.25	0.97	0.3364	0.5996
reciprocal interval	field	*capicola*	*vinacea*	-0.0006	0.000256	78.71	-2.34	0.0219	0.0565
		capicola	hybrid	0.000272	0.00022	78.77	1.24	0.219	0.4341
		hybrid	*vinacea*	-0.00087	0.000219	77.60	-3.98	0.0002	**0.0005**
	lab	*capicola*	*vinacea*	-0.00047	0.00027	76.49	-1.76	0.0827	0.1905
		capicola	F1	-0.0001	0.000245	76.53	-0.40	0.6896	0.9153
		F1	*vinacea*	-0.00057	0.000202	76.56	-2.83	0.0059	**0.016**
overall peak frequency	field	*capicola*	*vinacea*	140.51	18.8622	77.55	7.45	<.0001	**<.0001**
		capicola	hybrid	70.2335	16.2058	77.57	4.33	<.0001	**0.0001**
		hybrid	*vinacea*	70.2788	16.1878	77.22	4.34	<.0001	**0.0001**
	lab	*capicola*	*vinacea*	137.16	20.0162	76.89	6.85	<.0001	**<.0001**
		capicola	F1	-77.8554	18.1682	76.90	-4.29	<.0001	**0.0002**
		F1	*vinacea*	59.3078	14.9884	76.91	3.96	0.0002	**0.0005**
ln overall trill amplitude	field	*capicola*	*vinacea*	1.9141	0.1685	78.39	11.36	<.0001	**<.0001**
		capicola	hybrid	1.1529	0.1448	78.43	7.96	<.0001	**<.0001**
		hybrid	*vinacea*	0.7612	0.1444	77.58	5.27	<.0001	**<.0001**
	lab	*capicola*	*vinacea*	1.4849	0.1783	76.77	8.33	<.0001	**<.0001**
		capicola	F1	-1.1468	0.1618	76.81	-7.09	<.0001	**<.0001**
		F1	*vinacea*	0.3381	0.1335	76.82	2.53	0.0134	**0.0353**
overall trill rate	field	*capicola*	*vinacea*	4.9298	1.4746	78.63	3.34	0.0013	**0.0036**
		capicola	hybrid	1.9105	1.2671	78.68	1.51	0.1356	0.2929
		hybrid	*vinacea*	3.0193	1.2626	77.57	2.39	0.0192	**0.0498**
	lab	*capicola*	*vinacea*	4.0297	1.5574	76.51	2.59	0.0116	**0.0307**
		capicola	F1	-1.7653	1.4138	76.55	-1.25	0.2156	0.4285
		F1	*vinacea*	2.2644	1.1664	76.58	1.94	0.0559	0.134
DTW PC1	field	*capicola*	*vinacea*	0.5121	0.03523	78.00	14.54	<.0001	**<.0001**
		capicola	hybrid	0.2458	0.03005	78.00	8.18	<.0001	**<.0001**
		hybrid	*vinacea*	0.2664	0.03005	78.00	8.87	<.0001	**<.0001**
	lab	*capicola*	*vinacea*	0.4697	0.03747	78.00	12.49	<.0001	**<.0001**
		capicola	F1	-0.2853	0.03401	78.00	-8.39	<.0001	**<.0001**
		F1	*vinacea*	0.1826	0.02806	78.00	6.51	<.0001	**<.0001**

by the group Coefficients of Variation of the six parameters and the DTW results (Table 5.4). For the parameters Coo to Coo and Interval the F1 hybrids between individual variation is lower than in the parental species *vinacea*. For the Overall Trill Amplitude it is higher than both parental species (Table 5.4). Within individual variation in F1 hybrids is also comparable to that in the parental species, except for Overall Trill Amplitude and Trill Rate where the variation is slightly higher in F1 hybrid individuals (mean Coefficients of Variation in Table 5.5).

Field hybrid and parental species vocalizations

Field hybrids also have an intermediate mean value compared to the parental species and span the range between them for all the parameters (except for the parameter Interval, Table 5.4 and Figures 5.1, 5.2 and 5.3). Field hybrids differ significantly from both parental species recorded in the field in Coo to Coo, Coo, Overall Peak Frequency, Overall Trill Amplitude and DTW PC1 (Table 5.3). For Interval and Overall Trill Rate they differed only from *vinacea*. This suggests they are not only intermediate, but form a distinct vocal group from both parental species. This is in line with earlier findings (de Kort *et al.*, 2002a).

The variation between individuals is comparable to that in the parental species as shown by the group Coefficients of Variation of the six parameters and the DTW results (Table 5.4). For the parameters Overall Trill Rate and DTW PC1 the field hybrid between individual variation is lower than in the parental species *vinacea*. Within individual variation in field hybrids is also comparable to that in the parental species, except for Overall Trill Amplitude where the variation is higher in field hybrid individuals (mean Coefficients of Variation in Table 5.5).

F1 and field hybrid vocalizations compared

F1 and field hybrids from the natural hybrid zone do not differ in their vocal characteristics. With respect to the parental species, both groups are intermediate, span the whole range, are separate groups and have a generally comparable within and between individual variation. When they do not differ from the parental species, they do so in the same parameters (Interval and Overall Trill Rate) with respect to the same species (*capicola*; Table 5.3). The only difference is that the F1 hybrids do not differ significantly from *vinacea* for Coo and Overall Trill Rate while the field hybrids do (Table 5.3).

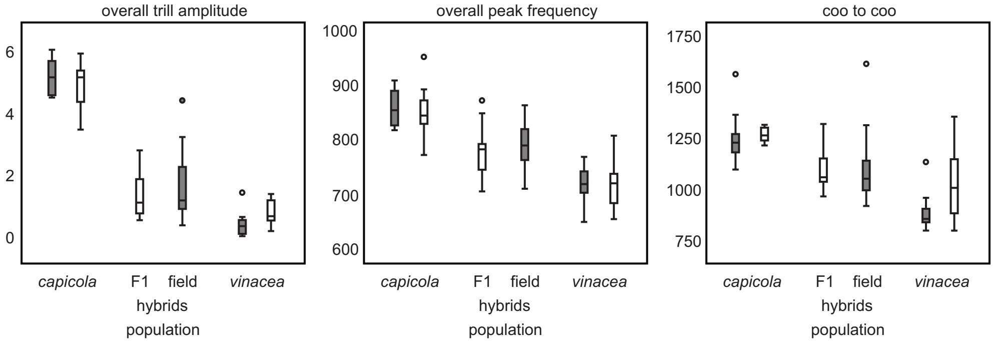


Figure 5.2 Box plots of song parameters per group: Overall Trill Amplitude, Overall Peak Frequency and Coo to Coo. Box plots represent the median, interquartile range and full ranges. Gray indicates groups were recorded in the field. White indicates groups were recorded in the lab. Samples sizes per group are: *capicola* field=10, *capicola* lab=7, F1=23, hybrid zone=21, *vinacea* field=10, *vinacea* lab=12. *Capicola* and *vinacea* differ in all acoustic parameters. There seem to be slight differences in these parameters depending on the recording location. F1 hybrids and field hybrids are intermediate in vocal characters between the two parental species and span the range between the two species.

Table 5.4 Means, standard deviation (σ), and Coefficients of Variation (CV) per parameter per group. Group means are averages of individual averages per group

population	location		coo to coo	coo	interval	overall peak frequency	overall trill amplitude	overall trill rate	DTW PC1
capicola	field	mean	1237.12	841.12	396.01	866.91	5.63	13.68	0.84
	N=10	σ	144.44	76.06	99.36	34.16	0.59	1.09	0.04
		CV	11.68	9.04	25.09	3.94	10.45	7.96	4.73
	lab	mean	1248.57	767.49	481.09	861.31	5.31	14.84	0.83
	N=7	σ	42.99	50.19	75.39	57.08	0.86	1.18	0.03
		CV	3.44	6.54	15.67	6.63	16.21	7.93	3.11
F1	lab	mean	1054.86	543.99	510.87	783.45	1.82	13.07	0.55
	N=23	σ	116.36	51.98	114.11	44.16	0.70	3.81	0.08
		CV	11.03	9.56	22.34	5.64	38.57	29.12	15.55
hybrid	field	mean	1055.03	588.78	466.25	796.66	2.07	11.77	0.60
	N=21	σ	180.52	74.92	153.11	40.69	1.04	2.67	0.09
		CV	17.11	12.73	32.84	5.11	50.36	22.81	15.90
vinacea	field	mean	821.73	487.75	333.99	726.45	0.90	8.73	0.33
	N=10	σ	111.41	41.97	102.80	33.08	0.41	5.21	0.09
		CV	13.56	8.61	30.78	4.55	45.48	59.74	26.02
	lab	mean	970.00	522.93	447.07	724.14	1.24	10.81	0.36
	N=12	σ	187.67	31.07	173.85	43.14	0.38	3.03	0.07
		CV	19.35	5.94	38.89	5.96	30.52	28.00	18.92

The between individual variation in both hybrid groups is comparable to that in the parental species. For both hybrid groups there are parameters in which the variation in *vinacea* is higher. The within individual variation in both hybrid groups is also comparable to that in the parental species. For the parameter Overall Trill Amplitude the variation in the hybrid groups is higher than in the parental species. For the parameter Overall Trill Rate, the variation is higher in the F1 hybrids than in the parental species. For the field hybrids, the variation in this parameter is comparable to the variation in the parental species.

F1 hybrids: reciprocal crosses and similarities between brothers

F1 hybrids from reciprocal crosses do not differ in their vocal characteristics (Table 5.6). F1 brothers were not more similar to each other than to unrelated individuals (Table 5.6). Both Cross type and Father were not significant in the linear mixed models. For the Coo parameters, Father

Table 5.5 Means of individual Coefficients of Variation per group

population	location (n)	coo to coo	coo	interval	overall peak frequency	overall trill amplitude	overall trill rate	DTW PC1
capicola	field (10)	4.97	4.56	26.31	2.57	16.06	13.27	4.70
	lab (7)	8.65	8.43	21.23	2.10	14.65	10.98	6.84
F1	lab (23)	12.47	6.74	20.44	2.07	27.01	23.99	13.26
hybrid	field (21)	11.33	5.77	23.06	2.22	32.33	25.23	8.23
vinacea	field (10)	7.27	8.13	31.65	2.34	19.14	19.21	12.49
	lab (12)	13.33	5.35	25.75	2.94	20.30	18.53	13.13

Table 5.6 Effect of father and cross type on overall vocal parameters in F1 hybrid males. df, degrees of freedom. Bold values indicate significant *P* values.

parameter	effect	numerator df	denominator df	F	*P*
reciprocal coo to coo	father	12	9.927	1.01	0.5036
	cross type	1	20.99	1.15	0.2951
coo	father	12	10.060	4.29	**0.0137**
	cross type	1	21.04	1.35	0.2575
reciprocal interval	father	12	9.911	1.02	0.4923
	cross type	1	20.95	3.94	0.0605
overall peak frequency	father	12	9.989	1.49	0.2690
	cross type	1	21.01	1.58	0.2232
overall trill amplitude	father	12	10.000	0.75	0.6853
	cross type	1	21.01	0.13	0.7261
overall trill rate	father	12	9.981	0.96	0.5326
	cross type	1	20.97	0.06	0.8097
DTW PC1	father	12	10	1.07	0.4664
	crosstype	1	21	0.21	0.6498

was significant. This concerns an individual that only had one F1 son, and this son was very different from all the other individuals (outlier) which caused the significant Father effect.

Discussion

Laboratory-bred F1 hybrids between the dove species *vinacea* and *capicola* have vocal characteristics that are similar in range and variation to natural hybrids in Uganda. This suggests that the whole range of possible vocalizations is achieved within one generation of interbreeding, i.e. individuals may sound like *vinacea*, *capicola* or be intermediate between the two, and that hybrid individuals from the same type of cross may vary greatly with respect to their vocalizations. F1 lab-bred hybrid vocalizations are intermediate and span the whole range between *vinacea* and *capicola* as do field hybrids. In general, both groups show a comparable variation between individuals to that of the parental species in their vocal characteristics. F1 hybrids from the two different reciprocal crosses do not differ in their vocal characters. Taken together, this suggests that either the natural hybrid zone consists only of F1s or that further interbreeding (F2 and backcrossing) does not increase vocal variation much. We believe the latter is the case, as our genetic analyses (Chapter 2) have shown that introgression into the *vinacea* population takes place, which indicates that backcrossing is occurring and possibly breeding between hybrids.

The seemingly continuous variation of vocalizations ranging from one parental species to those of the other in both the F1 and field hybrid populations may indicate a type I genetic architecture for dove vocalizations in which there are many genes of small effect (Templeton 1981). A large variation is not expected in F1 hybrids as all individuals are expected to be heterozygotic at most loci (Price 2008). In our doves, the F1 hybrids are variable between individuals, but overall we do not find a higher variation than in the parental species. Recombination in hybrids may lead to a higher variation between hybrid individuals than in the parental species but apparently this

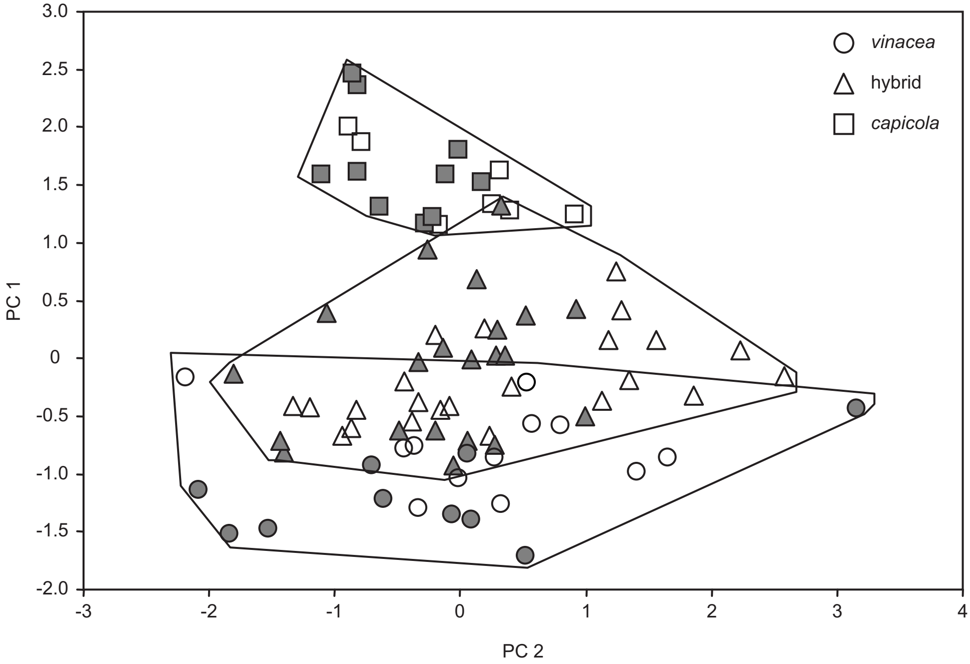


Figure 5.3 Scatter plots of the first two factors the Principal Component Analysis; the first (PC1) and second (PC2) extracted components are shown. Each dot represents an individual. Gray indicates groups were recorded in the field. White indicates groups were recorded in the lab. Samples sizes per group are: *capicola* field=10, *capicola* lab=7, F1=23, hybrid zone=21, *vinacea* field=10, *vinacea* lab=12. Polygons were made around *vinacea*, *capicola* and hybrid individuals. *Vinacea* and *capicola* individuals do not overlap in vocal characters. Both F1 and field hybrids are found in the parameter space between the parental species. There are individuals that resemble one of the parental species, individuals that are intermediate between the two species and individuals that do not seem to resemble either species.

is not necessarily always the case as we have shown. Work on laboratory-reared hybrids in non-songbirds has shown a higher variability in F1 hybrids than in parental species for vocal parameters (Collins & Goldsmith, 1998; Deregnaucourt *et al.*, 2001). Within individuals F1 hybrids and field hybrids do not seem to be more variable than the parental species. It is hard to predict what to expect for within-individual variation in hybrids.

For a polygenic trait, the variation is expected to be higher in F2 than F1 generations because of recombination and segregation (Shaw 1996; Price 2008). Individuals from the natural hybrid zone in the field are likely to be products of multiple generations of crossings between pure species and hybrids. The variation in field hybrids may therefore be higher than in the F1 population. Our data suggest this not to be the case. However, we cannot exclude that our comparison is affected by the fact that field and F1 hybrids were recorded under different conditions (field vs. lab). But when each group is compared with respect to the parental species they do not seem to be more variable than the parental species (between individual variation). The variation in field hybrids and parental species has been found to be of similar magnitude in other non-songbirds (Gee 2005), grasshoppers (Gottsberger & Mayer 2007) and crickets (Shaw 1996). Even though differences in vocalizations may be largely genetically determined, a higher genetic variability due to recombination in hybrids may not necessarily lead to a higher vocal variation between individuals. This may depend on the genetic divergence between these hybridizing species (around 1 million years for those mentioned above) rather than be a general phenomenon of hybridizing species with genetically determined acoustic signals. A 2.5% mtDNA divergence between our parental species (Johnson *et al.* 2001) may not be enough for them to be homozygous at all loci controlling vocal characters leading to an F1 generation that is not more variable than the parental species. Further hybridization may not increase the variation much for the same reason.

F1 males from crosses between *vinacea* females and *capicola* males and the reciprocal crosses did not differ in their vocal characters. Sex linkage of acoustic signals is therefore absent in these species. This is consistent with what has been found in quail (Deregnaucourt *et al.* 2001). Sex linkage of acoustic signals has been found a few times in different insect species (Bentley & Hoy, 1972; Shaw, 1996; Vedenina & von Helversen, 2003; Gottsberger & Mayer, 2007). If vocalizations are not sex-linked, there can not be selection on vocal signals favouring one cross type over the other and the products of both crosses will have comparable fitness. Differential introgression of mitochondrial DNA from one species to the other (as observed in Chapter 2), will therefore also not be particularly favoured based on vocal characters.

There seem to be no obvious ecological differences between *vinacea* and *capicola*. Overall, they occupy a similar habitat and seem to have a similar diet (Urban *et al.* 1986). Both species eat seeds and invertebrates, whether they consume different species in their respective allopatric populations is unknown. 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 width of this hybrid zone will mostly be determined by hybrid fitness as opposed to interactions between parental species and their fitness in different habitats (Price 2008). Selection against hybrids is often assumed to complete speciation through reinforcement of pre-mating barriers. In our species, F1 hybrids are viable (see also Chapter 2) and likely to be fertile as we had one incidence of an F2 hatching. Even so, selection against hybrid signals could cause fitness reduction in hybrids. Aberrant hybrid signals may be dysfunctional in territorial and mate attraction leading to 'behavioural sterility' (Coyne & Orr 2004; Gottsberger & Mayer 2007; Price 2008).

Individuals with different vocalizations may experience different fitness consequences. F1 hybrids that have vocalizations that are strictly intermediate between *vinacea* and *capicola*, may experience reduced fitness. In playback experiments with natural hybrid signals, we found that within the hybrid zone, territorial males respond to field hybrid vocalizations covering the whole range as they do to parental species vocalizations. In the allopatric parental populations however, the response to field hybrid signals was reduced when compared to the response to the conspecific signal (Chapter 3). This suggests that within the hybrid zone, hybrids with all sorts of vocalizations are capable of obtaining and defending a territory and possibly achieve reproduction. Outside the hybrid zone, those males with vocalizations that differ from the local parental species may have less success, and not be able to establish a territory. However, hybrids resembling one of the parental species in vocal characters may obtain territories outside the hybrid zone, and not suffer a loss of fitness.

It is unclear how females, of both parental species and hybrids, respond to hybrid signals. Female response may differ from male response as they have more to lose from choosing the 'wrong' male (Searcy & Brenowitz 1988). If female hybrids respond as males do, they will accept hybrids of all sorts as mates. Hybrid females have been shown to prefer hybrid male signals in frogs (Doherty & Gerhardt 1984) and crickets (Shaw 2000). If this is the case, hybrid males may do well within the hybrid zone, but not outside it. Parental species females have been found to respond to hybrids as to conspecifics (Deregnaucourt & Guyomarc'h 2003) and form pairs with them (Gee 2005) within the hybrid zone. If females of the parental species in allopatric populations also accept hybrids as mates, hybrids may be able to establish themselves outside the hybrid zone. Usually females of the parental species are expected to show a preference for conspecific signals (Bridle *et al.* 2006) and may accept hybrid males that sound like a conspecific male, allowing for introgression. An important factor determining both male and female response to male vocalizations is whether responses are learned or mostly genetically determined (Chapter 4).

The pattern of F1 vocalizations we found in *Streptopelia* doves is congruent with what has been found in other non-songbird species (Collins & Goldsmith 1998; Ceugniet *et al.* 1999; Deregnaucourt *et al.* 2001) and some other vertebrate species (Doherty & Gerhardt 1983, 1984) in which F1 individuals are intermediate and can resemble the parental vocalizations. Therefore, this inheritance mode may be common in more vertebrate species. It is likely that in vertebrate species that do not learn their vocalizations, acoustic signals have a polygenic basis. This may lead to a large range of vocalizations in the F1 hybrid generation. This entails that in these species, F1 hybrid vocalizations may allow further introgression because there will always be some individuals that resemble one of the parental species. Once there is hybridization, hybrid aberrant signals will not necessarily prevent further introgression.

Studies comparing F1 hybrid signals to natural hybrid signals in the field are valuable because they give an integrated picture of the effect of hybridization on acoustic signals. The laboratory setting allows for testing of possible sex-linked, genetic and the general effects of hybridization on vocalizations. Alternatively, analyzing hybrids in the field under natural circumstances and selection pressures, adds to the general picture of the causes and consequences of hybridization for vocal characters and gives us an idea of the fitness consequences they bear. Our study demonstrates that within one generation of interbreeding the whole range of variation between parental species vocalizations can be achieved and that there are no sex-linked effects on the vocalizations. This gives better insight into how vocal variation in field hybrids arises and allows us to conclude that it is not necessarily due to multiple generations of interbreeding. A large range in hybrid vocalizations, including overlap with parental species, may allow for further introgression between species rather than preventing it. This suggests that in many contact events, hybrid vocalizations may contribute to "despeciation" (Grant & Grant 2006) rather than to speciation. Hybrid zones may therefore not disappear, resulting in complete speciation, but remain as a stable factor between two species.

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