

Modelling Perception **with Artificial** **Neural Networks**

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Evolutionary diversification of mating behaviour: using artificial neural networks to study reproductive character displacement and speciation

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9.1 Introduction

When species with similar sexual signals co-occur, selection may favour divergence of these signals to minimise either their interference or the risk of mis-mating between species, a process termed reproductive character displacement (Howard, 1993; Andersson, 1994; Servedio & Noor, 2003; Coyne & Orr, 2004; Pfennig & Pfennig, 2009). This selective process potentially results in mating behaviours that are not only divergent between species that co-occur but that are also divergent among conspecific populations that do and do not occur with heterospecifics or that co-occur with different heterospecifics (reviewed in Howard, 1993; Andersson, 1994; Gerhardt & Huber, 2002; Coyne & Orr, 2004; e.g., Noor, 1995; Saetre *et al.*, 1997; Pfennig, 2000; Gabor & Ryan, 2001; Höbel & Gerhardt, 2003).

An oft-used approach to assessing whether reproductive character displacement has occurred between species relies on behavioural experiments that evaluate mate preferences from populations that do and do not occur with heterospecifics (sympatry and allopatry, respectively). In such experiments, individuals are presented the signals of heterospecifics and/or conspecifics to assess whether allopatric individuals are more likely to mistakenly prefer heterospecifics than are sympatric individuals (reviewed in Howard, 1993). The expectation is that individuals from sympatry should preferentially avoid heterospecifics, whereas those in allopatry should fail to distinguish heterospecifics from conspecifics (presumably because, unlike sympatric individuals, they have not been under selection to do so). Such patterns of discrimination have been observed, and they provide some of the strongest examples of reproductive character displacement (reviewed in Howard, 1993).

A problem with this approach, however, is that allopatric females also may discriminate against heterospecifics depending on how heterospecific signals vary relative to preferred conspecific signals (Rodriguez *et al.*, 2004; Pfennig & Ryan, 2007). If heterospecific signals possess characters that are disfavoured by allopatric females, heterospecific signals may be selected against even if they have never been encountered. For

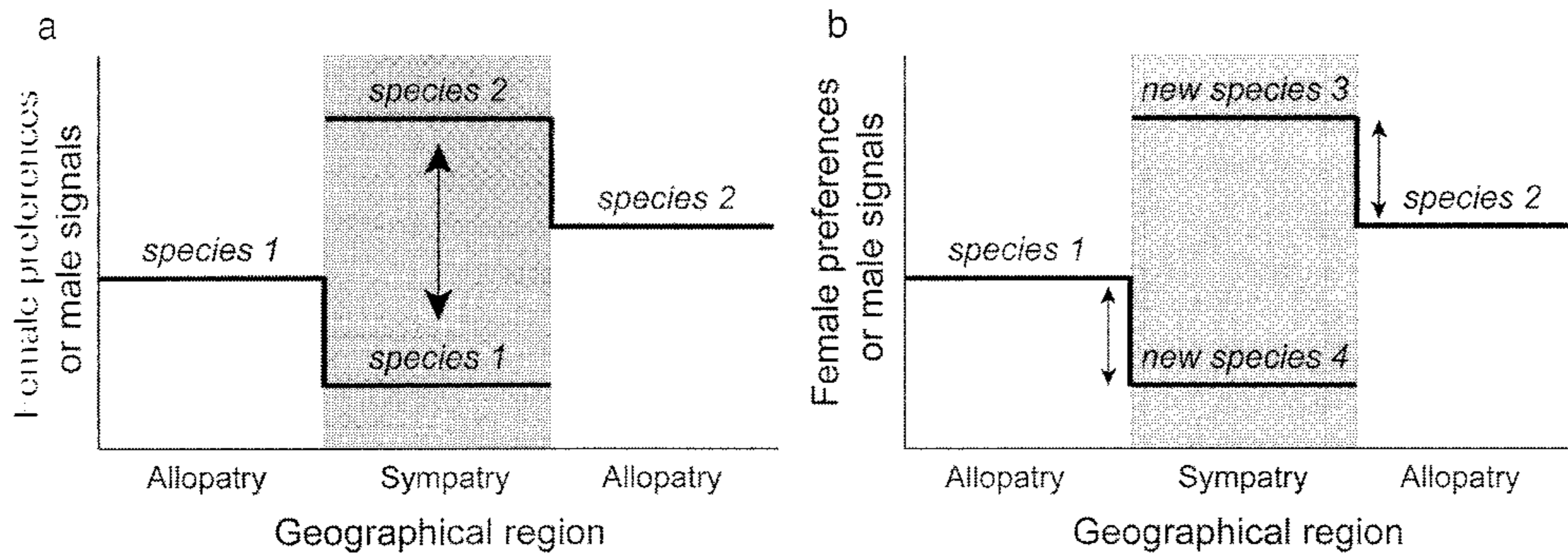


Figure 9.1. Reproductive character displacement can both finalise and initiate speciation. (a) Character displacement may finalise the speciation process by directly promoting the evolution of reproductive isolation between populations. When populations that have diverged in allopatry come together in only part of their geographical range (indicated by the shading), selection to minimise reproductive interference or hybridisation may exaggerate differences in mating behaviours (indicated by the doubled-headed arrow). Such divergence thereby enhances reproductive isolation between existing or incipient species. (b) Character displacement may also initiate speciation by indirectly promoting the evolution of reproductive isolation between conspecific populations. In particular, an indirect consequence of character displacement is that sympatric individuals will evolve different mate preferences and/or mate attraction signals than allopatric conspecifics. If male signals or female preferences diverge to the point that sympatric and allopatric individuals do not recognise each other as potential mates, reproductive isolation results. This process may eventually promote the formation of two new species (indicated here as ‘new species 3’ and ‘new species 4’). Modified from Pfennig & Pfennig (2009) and Pfennig & Rice (2007).

9.2 Using artificial neural networks to study character displacement

Here, we describe our previously published work (Pfennig & Ryan, 2006, 2007) in which we used artificial neural networks to mimic the evolution of conspecific recognition in response to different heterospecific interactions. Artificial neural networks, also called connectionist models, consist of computational units (‘neurons’) that can stimulate or inhibit each other and are connected into networks. These interconnected units (networks) can simulate behaviour in response to an input and have been likened to the nervous system in function (Enquist & Ghirlanda, 2005).

Artificial neural network models are a potentially powerful tool for examining how mating behaviours diversify and the role of this diversification in speciation. Populations of networks can be generated that evolve mating behaviours under different selective contexts or that undergo different signalling interactions. Such models thereby allow for an understanding of how individual behaviours contribute to larger evolutionary patterns of diversification and speciation.

For example, neural network simulations have provided key insights into how both historical contingency and other species in the signalling environment influence how conspecific signals are recognised (Phelps & Ryan, 1998, 2000; Ryan & Getz, 2000;

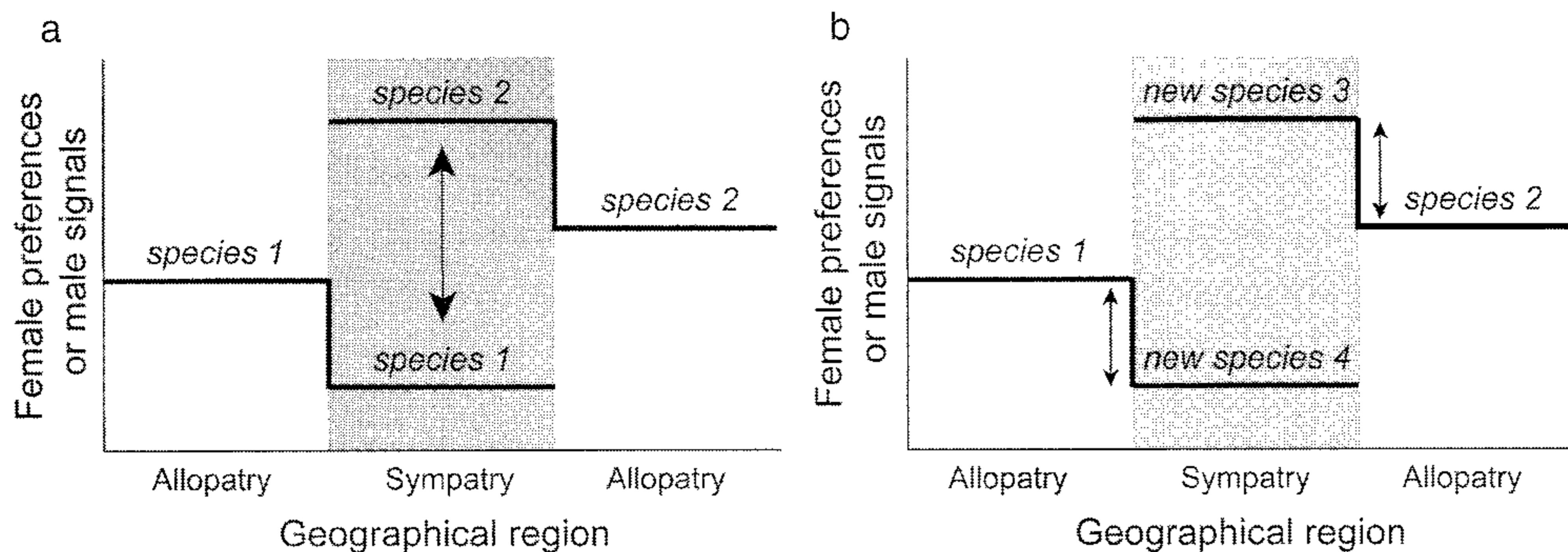


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Phelps *et al.*, 2001; Ryan *et al.* 2001). Indeed, Phelps & Ryan (1998, 2000) showed how the training of artificial neural networks could be used to mimic the past evolutionary history of frog calls to demonstrate how history influences recognition patterns of real female túngara frogs (*Physalaemus pustulosus*). Although they simulated a specific system, these studies came to the general conclusion that computational strategies used in mate recognition by current species are importantly influenced by the recognition strategies used by their ancestors. Such studies illustrate how artificial neural networks can be used as tools for better understanding evolutionary patterns and processes.

Artificial neural networks are particularly useful for investigating how mate recognition evolves among populations that co-occur with different heterospecifics. In natural systems, the presence of heterospecifics often covaries with changes in habitat or population evolutionary history. These factors can generate patterns that are consistent with, but that are not actually the result of, character displacement. Simulations with artificial neural networks provide a means for focusing on how signallers and receivers coevolve owing to heterospecific interactions in order to clarify the predictions that can be tested empirically. Indeed, as we note in our Discussion, simulations with artificial neural networks can identify how some empirical approaches may be overly conservative in their approach to character displacement.

9.3 The model

We mimicked a system in which males use pulsatile calls to attract females as mates (as occurs in many anuran and insect systems; Gerhardt & Huber, 2002). Although we simulated species recognition for acoustic signals, our results likely can be generalised to other sensory modalities. We based elements of our model on a naturally occurring spadefoot toad species, *Spea multiplicata*. As in many species, *S. multiplicata* occur with different species in different parts of their range in the southwestern region of the USA (Stebbins, 2003). In the eastern part of their range, for example, they co-occur with a congener, *S. bombifrons*. In the western part of their range, they occur with another spadefoot toad, *Scaphiopus couchii*. In still other populations they are the only spadefoot species present. These distributional patterns make *S. multiplicata* an excellent system for assessing how female behaviours evolve among disparate populations. We therefore used elements of this system to inform a model aimed at investigating how heterospecific interactions affect the evolution of female mate preferences among different populations. Because we did not model the spadefoot system explicitly, however, many features of our model differ markedly from the spadefoots' natural history. Our goal was not to mimic the spadefoot system per se, but to use this system to guide the modelling efforts described below.

We generated three population types consisting solely of networks belonging to the same species, 'species A'. Depending on the population type, the networks evolved conspecific recognition of advertisement signals of species A in the face of no heterospecific signals, or when faced with discrimination of signals from their own species

versus signals from other species. In the first type, networks were trained to recognize conspecific acoustic stimuli in the presence of no heterospecific signals. In the second type, networks were trained to recognize conspecific acoustic stimuli in the presence of heterospecific signals. In the third type, networks were trained to recognize conspecific acoustic stimuli in the presence of heterospecific signals, but the heterospecific signals were filtered out of the input. In all cases, the networks were trained to recognize conspecific acoustic stimuli in the presence of heterospecific signals, but the heterospecific signals were filtered out of the input. In all cases, the networks were trained to recognize conspecific acoustic stimuli in the presence of heterospecific signals, but the heterospecific signals were filtered out of the input.

We used the neural network toolbox in Matlab (The Mathworks, Natick, MA) to simulate a network of 35 input neurons, each receiving a different frequency in the input signal. The input signal was passed forward to a single layer of 35 neurons, which were then passed forward to a single layer of 35 neurons. The output of this layer was then passed forward to a single layer of 35 neurons. The output of this layer was then passed forward to a single layer of 35 neurons. The output of this layer was then passed forward to a single layer of 35 neurons. The output of this layer was then passed forward to a single layer of 35 neurons.

The activity of each neuron was calculated as a hyperbolic tangent function of the weighted sum of its inputs. The weights of the connections from the input neurons to the hidden layer neurons were initialized to random values. The weights of the connections from the hidden layer neurons to the output neurons were initialized to random values. The weights of the connections from the input neurons to the output neurons were initialized to random values. The weights of the connections from the hidden layer neurons to the output neurons were initialized to random values.

where \mathbf{p} was a 35 × 1 vector representing the signal matrix, \mathbf{w} was a 35 × 35 matrix representing the weights of the connections between the input neurons and the hidden layer neurons, and \mathbf{b} was a 35 × 1 vector representing the bias of the hidden layer neurons. The weights of the connections from the hidden layer neurons to the output neurons were initialized to random values. The weights of the connections from the input neurons to the output neurons were initialized to random values.

versus signals from one of two heterospecific species. In particular, in one population type, networks were selected for the ability to discriminate representations of conspecific acoustic stimuli of ‘species A’ from white noise. The white noise stimulus controlled for the presence of a second stimulus and provided a means of assaying the networks’ recognition of a conspecific signal. We refer to this population type as ‘A’. This population mimics the evolution of conspecific recognition in the absence of heterospecifics. In the second population type, ‘species A’ networks evolved to discriminate between conspecific stimuli of ‘species A’ and stimuli of a heterospecific, ‘species B’. We refer to this population type as ‘AB’. Finally, in a third population type, networks evolved to discriminate between conspecific stimuli of ‘species A’, and stimuli from a second heterospecific, ‘species C’. We refer to this population type as ‘AC’. For a list of definitions and usage of key terms, see Table 9.1.

We used the standard Elman network (Elman, 1990) available in the neural network toolbox in Matlab (Demuth & Beale, 1997). The network architecture consisted of a layer of 35 input neurons that received the stimulus (each neuron responded to a different frequency in the signal; see below for details of signal properties) and then fed this input forward to a single hidden layer of 23 neurons. Activity from this hidden layer was then fed forward to a single output neuron (see below). Elman networks are particularly effective at decoding stimuli that are temporally structured (e.g. acoustic stimuli) because the Elman architecture includes recurrent connections within the hidden layer so that the neurons of the hidden layer feed back onto themselves (Elman, 1990; Demuth & Beale, 1997; e.g. Phelps & Ryan, 1998, 2000; Ryan & Getz, 2000; Phelps *et al.*, 2001). This recurrence permits the processing of information in a current time-step contingent on the information from a preceding time-step. Evolutionary simulations using similarly structured networks have been shown to predict female preferences for both conspecific and heterospecific male calls in túngara frogs (Phelps & Ryan, 1998; Phelps & Ryan, 2000; Phelps *et al.*, 2001).

The activity of the input layer was not weighted, and was determined strictly by the stimulus input. The stimulus was input over the course of 190 time steps, where each time step corresponded to a column, analogous to a slice of time, in the signal matrix (see below for description). The activity of the hidden layer, \mathbf{a}^1 , was determined using a hyperbolic tangent (tansig) transfer function that combined the activity and weights of connections from the input, the recurrent connections and a bias (notation here and below is that of Demuth & Beale, 1997):

$$\mathbf{a}^1(k) = \text{tansig}(\mathbf{I}\mathbf{W}^{1,1}\mathbf{p} + \mathbf{L}\mathbf{W}^{1,1}\mathbf{a}^1(k-1) + \mathbf{b}^1) \quad (1)$$

where \mathbf{p} was a 35×1 vector from the input layer corresponding to the k th column from the signal matrix. $\mathbf{I}\mathbf{W}^{1,1}$ was a 23×35 matrix, the elements of which constituted the weights of the connections between the input and hidden layer, $\mathbf{L}\mathbf{W}^{1,1}$ was a 23×35 matrix that constituted the weights of the recurrent connections of the hidden layer neurons, and \mathbf{b}^1 was a 23×1 bias vector (Demuth & Beale, 1997). Biases enable networks to represent relationships between a signal and output more easily than networks without biases (Demuth & Beale, 1997). The sizes of the bias vectors corresponded

Table 9.1. *Definitions and usage of key terms used throughout the chapter.*

Term	Definition and usage
Conspecific	Of the same species. The calls of three species are used in the simulations: <i>A</i> , <i>B</i> and <i>C</i> . Species <i>A</i> is the focal species for all simulations.
Heterospecific	Of a different species. For example, the calls of <i>B</i> are heterospecific calls for <i>A</i> .
Population	A group of 100 networks that undergo selection, mutation and evolution in response to different discrimination tasks. Three types of populations were generated for our simulations: <i>A</i> populations, in which networks were presented conspecific calls of species <i>A</i> versus white noise; <i>AB</i> populations in which networks were presented conspecific calls of species <i>A</i> versus heterospecific calls of species <i>B</i> ; and <i>AC</i> populations in which networks were presented conspecific calls of species <i>A</i> versus heterospecific calls of species <i>C</i> . Note that <i>A</i> , <i>AB</i> and <i>AC</i> populations are all conspecifics – they consist of networks of the same species (species <i>A</i>).
Replicate	A population that has undergone selection, mutation and evolution. Depending on the question that was being addressed, we generated either 20 or 30 replicates for each population type described above.
Sympatric	Of a population occurring with a given heterospecific species. Networks in the <i>AB</i> populations are sympatric with species <i>B</i> (but not species <i>C</i>); networks in the <i>AC</i> population are sympatric with species <i>C</i> (but not species <i>B</i>); networks in the <i>A</i> populations are not sympatric with any species.
Allopatric	Of a population that does not occur with a given heterospecific species. Networks in the <i>AB</i> populations are allopatric with species <i>C</i> (but not species <i>B</i>); networks in the <i>AC</i> population are allopatric with species <i>B</i> (but not species <i>C</i>); networks in the <i>A</i> populations are allopatric with both <i>B</i> and <i>C</i> .
Local	Of the same population type or replicate. In contrasting calls from different population types: calls from the <i>A</i> populations are local only for <i>A</i> populations; calls from the <i>AB</i> populations are local only for <i>AB</i> populations; and calls from the <i>AC</i> populations are local only for <i>AC</i> populations. In contrasting calls from different replicates <i>of the same population type</i> , local calls are those of a single population.
Foreign	Of a different population type or replicate. In contrasting calls from different population types: calls from the <i>A</i> populations are foreign to both <i>AB</i> and <i>AC</i> populations; calls from the <i>AB</i> populations are foreign to both <i>A</i> and <i>AC</i> populations; and calls from the <i>AC</i> populations are foreign to both <i>A</i> and <i>AB</i> populations. In contrasting calls from different replicates <i>of the same population type</i> , foreign calls are those of a different replicate.

to the number of neurons in the hidden and recurrent layers (Demuth & Beale, 1997). The biases were subject to mutation and so could evolve in our simulations (see below). The hyperbolic tangent transfer function limits the output from the hidden layer to values ranging from -1 to 1 (Demuth & Beale, 1997).

The activity of the output neuron, \mathbf{a}^2 , was the result of a pure linear transfer function that combined the activity and connections to it with a bias:

$$\mathbf{a}^2(k) = \text{purelin}(\mathbf{LW}^{2,1}\mathbf{a}^1(k) + \mathbf{b}^2) \quad (2)$$

where $\mathbf{LW}^{2,1}$ was a 1×23 matrix that constituted the weights connecting the output neuron with the neurons of the hidden layer and \mathbf{b}^2 was a 23×1 bias vector. The pure linear transfer function calculated output by returning the value passed to it. Thus, there were no limits on output values.

The resulting output from each network was a vector of responses corresponding to each column in the signal matrix. We summed this vector to obtain a single scalar response measure to the entire signal matrix. Summing in this way was appropriate, as we had no a priori reason to weight the networks' responses to different time points in the signal differently. For further details and schematics of the network architecture see Demuth & Beale (1997) and Ryan & Getz (2000).

9.4 Simulating the evolution of conspecific recognition

We used a genetic algorithm to simulate the evolution of conspecific recognition. Networks underwent selection and mutation before being passed to the next generation. Our methods, which were similar to those of Ryan & Getz (2000), are described below.

For each population type, we created 100 networks consisting of the architecture described above. The matrix values used to specify each network were initially uniformly randomly generated with values constrained between -1 and 1 . We then presented to each network a conspecific stimulus and either a noise stimulus or one of two different heterospecific stimuli (the particular stimuli depended on the population in which the network 'resided'; see above). We defined the fitness of a network as the difference between its response to the conspecific stimulus and its response to the heterospecific stimulus. This fitness function results in higher fitness for those networks that are better able to discriminate between conspecifics and heterospecifics (i.e. those that maximise their responses to conspecifics while minimising their responses to heterospecifics). In nature, females must typically discriminate among courting males of different species (e.g. in a frog chorus males of different species could be calling simultaneously), so selection likely operates to maximise the likelihood of choosing the correct species while minimising the likelihood of selecting the wrong species (Reeve, 1989; Wiley, 1994). Because fitness cannot be negative (e.g. a female cannot have fewer than no offspring from a mating), negative fitness values were truncated to zero.

Using these fitness measures, we randomly selected the networks that were passed to the next generation. In particular, we selected 100 networks at random with replacement (i.e. the same network could be chosen more than once) from those networks in the preceding generation. The likelihood that a network was represented in the next generation was proportional to its fitness: networks with higher fitness had a higher likelihood of being chosen for the next generation than did networks with lower fitness.

Following this selection process, all networks that were selected to pass to the next generation underwent mutation (except a single network with the highest fitness in the previous generation). Values for the weights and biases of each network were chosen for mutation with a probability of 0.001. For those values that were chosen for mutation, we then added a random value between -0.5 and 0.5 to the existing value in each matrix element. Any values that exceeded 1.0 or were less than -1.0 were truncated to 1.0 and -1.0 respectively. Limits were set in order to mimic real biological systems in which neural activity has limits. Moreover, setting such limits is likely to make our findings conservative in that divergence in network behaviour becomes less, rather than more, likely. Previous work varying the nature of this mutation regime suggests that alterations do not appear to affect the general outcome of the simulations.

We used this general approach to ask two questions regarding the effects of heterospecific interactions on the evolution of female preferences. First we asked: how does character displacement affect mate preferences for conspecifics and discrimination against heterospecifics? To answer this first question, we examined how network preferences for conspecifics' signals and their ability to discriminate between conspecifics and heterospecifics would evolve differently among populations that varied in the nature of the heterospecific interactions they encountered. In these simulations, only the networks were allowed to evolve. By doing so, we could isolate the effects of heterospecific interactions on the evolution mate preferences.

Following the simulations to address the first question, we posed a second question: can reproductive character displacement initiate speciation? To address this question, we ran a second set of simulations. In these simulations, we allowed conspecific signals to evolve so as to evaluate how divergent preferences might contribute to diversification of conspecific signals. Our goal was to assess whether this divergence would tend to generate reproductive isolation among conspecific populations.

We therefore ran two sets of simulations. Below, we describe the stimuli presented to the networks, our application of the genetic algorithm described above, and our methods for evaluating network preferences in each set of simulations.

9.5 How does character displacement affect preferences for conspecifics and discrimination against heterospecifics?

9.5.1 Stimuli sets

The networks were presented pulsatile calls mimicking those possessed by many anuran and insect species. The calls were presented in a 35×190 frequency by time matrix in which the cell values ranged from 0 to 1 and represented amplitude of the signal at a given frequency and time (analogous to a sonogram). We synthesised the calls using a program written in Matlab that generated each call by combining randomly chosen values (see below) of four parameters: call duration (the length of the call in terms of matrix columns); call dominant frequency (the frequency in the call with the greatest energy,

Table 9.2. Mean (\pm SD) of call parameters for each species, measured in terms of matrix columns or rows. See text for description of how calls were generated. The values below for species A were used throughout the set of simulations in which only the networks evolved. In the simulations where male calls coevolved with network preferences, only the call parameters of A, but not B or C, were allowed to evolve. In these coevolutionary simulations, the values below for species A were used in the initial generation and are therefore the parameters of the 'ancestral A' calls. See Figure 9.4 for contrast of evolved A calls versus the ancestral A calls.

Call parameter	Species		
	A	B	C
Call duration (cols.)	62.6 (7.9)	9.1 (0.7)	62.4 (5.0)
Inter-call interval (cols.)	72.0 (1.7)	64.8 (0.9)	87.6 (4.7)
Call pulse rate (pulses/col.)	0.05 (0.01)	0.42 (0.05)	0.34 (0.02)
Dominant frequency (rows)	15.6 (1.2)	18.5 (1.2)	18.4 (1.5)

measured in terms of matrix rows); pulse rate (measured as number of pulses per matrix column); and inter-call interval (the number of matrix columns between the last column of the first call and the first column of the second call). This last parameter is a measure of calling rate; greater inter-call intervals result in slower call rates, whereas smaller inter-call intervals result in faster call rates.

Each call presented to a network was generated by randomly choosing a parameter value from the appropriate distribution for the conspecific or heterospecific calls. The distributions used for these parameter values were those of three naturally co-occurring spadefoot toads (*S. multiplicata*, *S. bombifrons* and *Sc. couchii*) from southeastern Arizona, USA (Pfennig, 2000). Once these parameter values were chosen, the duration of the call was shortened to 13% of its original length and the inter-call interval was shortened to approximately 5% of its original value, so that the duration of the longest possible call sequence would fit within the matrix presented to the networks. Pulse rate values were not altered from those chosen from the natural distributions; we report measures of pulse rate herein in terms of columns of the stimulus matrix, which represent time. We multiplied this pulse rate by the shortened call duration to obtain the number of pulses that would make up each call. Pulse length therefore varied within and between species, and was dependent on the combined parameters of pulse rate and call duration. Dominant frequency was converted to row values of the matrix. The resulting distribution of the call parameters measured in terms of rows and columns of the matrix are given in Table 9.2.

Using the randomly chosen parameters, each call was synthesised by initially generating a single pulse. To do so, a value of 1 (the maximum value of amplitude in the signal matrix) was assigned in the row corresponding to the dominant frequency of the call at the column corresponding the onset of the call (the onset of the call in the call matrix was randomly determined). The values in the following columns then degraded from 1

exponentially, and the values in the adjacent rows degraded exponentially from the values in the columns. This pattern thereby created a triangular pulse. The pulse was then repeated as appropriate in subsequent columns and rows of the matrix to generate a single call with the appropriate duration and pulse rate. A gap of silence (where values within the columns were set to 0) equivalent to the inter-call interval followed the call, at the end of which we appended a single pulse to indicate the onset of a second call.

The white noise stimuli presented to networks in the *A* populations were generated by assigning uniform random values ranging from 0 to 1 in a matrix that was the same size as that of the male calls. Moreover, after generating the male calls as described above, we also added noise to calls to simulate communication in a noisy environment. We did this by adding uniform random values ranging from 0 to 1 to the elements in each call matrix; resulting values greater than 1 were truncated to 1. By adding noise to the call stimuli, we ensured that all populations experienced white noise and therefore any differences that arose would not be an artefact of the noise stimulus. The amplitude of all stimuli presented to the networks was standardised so that they were equal in total amplitude.

Although some individual call characters were similar between species *A* (the conspecific species) and at least one of the heterospecific species (Table 9.2), the multivariate means of the call parameters were significantly different among all three species based on a sample of 20 randomly generated calls for each species (Wilks' $F_{6,110} = 192.08$, $p < 0.001$). Indeed, a discriminant analysis showed that all calls could be reliably assigned to the correct species based on their characteristics, a pattern that differed significantly from random expectation (log-likelihood ratio $\chi^2_4 = 131.83$, $p < 0.001$). By using calls that could be discriminated statistically from one another based on a combination of the calls' characters, we created a situation in which the impact of heterospecific interactions on the evolution of mate preferences should have been minimal. If heterospecific calls are sufficiently different from conspecifics, females can possibly identify conspecifics based solely on the variation of conspecific calls rather than the variation of conspecific calls relative to that of heterospecific calls (Patterson, 1985).

9.5.2 Simulations, testing and analyses of networks' responses

The above stimuli were presented to the networks, and using the genetic algorithm described above, the selection and mutation process was repeated for 1000 generations. We then replicated the entire procedure 20 times for each population type. Both the mean population fitness and maximum fitness for all replicates reached a plateau prior to generation 1000.

Following the above simulations, we selected the single network with the highest fitness from the last generation in each population type from each of the 20 replicates. To determine the nature of selection on each call parameter by networks from the three different population types, we tested each network with a series of conspecific calls in which each call parameter was systematically varied while all the other call characters were held constant. In particular, for each conspecific call character we generated a series

of calls in which each character took on values ranging from 3.5 standard deviations below the mean for that character to 4.0 standard deviations above the mean in 0.5 standard deviation intervals. All other call parameters were fixed at the mean values for those traits. Thus, for each of the four call characters we generated 15 variants. In addition to these call variants, we also presented the networks with a call in which all the call characters were set at the mean values for all four traits constituting a conspecific species A call (Table 9.2). Thus, the networks were presented a total of 61 different calls in this analysis.

We averaged the responses of the 20 networks from each population type to each of the call variants, and standardised these data so that they would be comparable across the different call parameters. We then regressed the network responses on the variation of each call character using a second-order polynomial regression (Sokal & Rohlf, 1995). If the second-order regression coefficient was not significant, that term was dropped from the model and a linear regression used. This analysis allowed us to determine the nature of selection on each call character exerted by the networks in each of the three populations (Falconer & Mackay, 1996; Conner & Hartl, 2004). Essentially, this analysis resulted in population level 'preference functions' for each call character in each population (Gerhardt, 1991; Wagner, 1998; Höbel & Gerhardt, 2003; Rodriguez *et al.*, 2004).

To evaluate whether networks in the different populations diverged in their preferences for male traits, we performed the following analysis. First, using standardised data, we regressed each network's response onto the systematic variation in each trait using second-order polynomial regression (Sokal & Rohlf, 1995). This gave us each network's preference function for each call character (Wagner, 1998). This analysis generated eight total regression coefficients (i.e. one first- and one second-order regression coefficient for each of four call characters) for each network in each population. We used principal component analysis (Sokal & Rohlf, 1995) to reduce the eight regression coefficients to a more manageable variable set. We then used MANOVA (Zar, 1984) to determine if the populations were significantly different in their values of these principal components. By doing so, we evaluated whether networks from the different populations differed significantly in their preference functions for, and therefore the pattern of selection they might exert on, conspecific male traits.

We next assayed whether the networks diverged in their ability to discriminate between conspecific and heterospecific calls. In one set of tests, we presented each network with a randomly generated conspecific call and a randomly chosen call of species C. In a second set of tests, we presented each network with a randomly generated conspecific call and a randomly generated call of species B. In each set of tests, we presented each network with 100 pairs of calls. In each pairing we scored a network as preferring a stimulus when it had a higher response to that stimulus. We then calculated the proportion of pairings in which the network showed preference for the conspecific stimulus. We used these individual measures to calculate population means. These means were compared among the populations with ANOVA and Tukey-Kramer HSD multiple comparisons tests to determine if the populations differed in their ability to

discriminate between conspecific and heterospecific males. In each population we also tested whether the networks significantly preferred the conspecific male. To do so, we tested whether the population mean preference for or against conspecific calls was significantly different from 50%, which is the null expectation if the networks were random in their preference of conspecifics versus heterospecifics. In all analyses described above, the data met parametric assumptions.

9.6 Can reproductive character displacement initiate speciation?

9.6.1 Stimuli sets

To determine whether reproductive character displacement can initiate speciation, we generated call stimuli as described above. In this set of simulations, however, we allowed the conspecific male calls, but not the heterospecific calls, to evolve in our simulations. Heterospecific calls were not allowed to evolve, because there would be no reason to expect coevolution between preferences in one species and calls of another species. Our simulation assumes that the heterospecific calls are at an evolutionary equilibrium. Further work is required to understand how evolutionary dynamics in one species affects coevolutionary dynamics between preferences and sexual signals in another species.

To allow conspecific calls to evolve, at each generation, the 100 conspecific calls that had been presented to the 100 networks passed to the next generation were also passed to the next generation (i.e. the calls represented the sires and the networks represented the dams of the next generation's offspring). From these calls we obtained the mean and standard deviation for each call parameter. These new distributions were then used to generate the calls (as described above) in the subsequent generation. Thus, in each generation, calls were randomly generated from the distribution of calls of the 'sires' in the previous generation. Calls were not pooled across replicates. Each replicate represented an independent evolutionary simulation of both species recognition and signal evolution.

For each replicate, we calculated the mean call parameters of the 100 calls in the final generation. These means were combined into a single data set along with call parameters of 30 randomly generated calls for each of the ancestral *A* population, and *B* and *C* species. The randomly generated ancestral and heterospecific calls served as samples of these calls types.

We analysed these data using a principal component analysis, which generated two principal components that described the joint variation in the four parameters. Both principal components had eigenvalues greater than 1. The first explained 52.8% of the variation in the advertisement calls, whereas the second explained 26.1% of the variation. We used these principal component values to compare the calls among the *A*, *AB* and *AC* populations based on the combined variation in the four call parameters. Because the data did not meet parametric assumptions, we compared each principal component among pairs of populations using Wilcoxon rank sums tests. We used a Bonferroni corrected alpha level of 0.017 in these multiple comparisons (Sokal & Rohlf, 1995).

9.6.2 Simulations, testing and analyses of networks' responses

The above stimuli were presented to the networks, and using the genetic algorithm described above, the selection and mutation process was repeated for 200 generations. We then replicated the entire procedure 30 times for each population type. The mean population fitness and maximum fitness for all replicates reached a plateau by generation 200.

We selected the network with the highest fitness from every 8th generation up through to the last generation in each population type from each of the 30 replicates. We tested these networks for preferences of their own conspecific calls versus the heterospecific (or noise) stimulus with which they coevolved. More critically, we also assayed the responses of these networks to advertisement calls of their own population (*local calls*) versus those of the two alternative populations (*foreign conspecific calls*). In the tests described below, we used the male call distributions from the networks' own generation.

To test the networks' preferences for local calls versus the heterospecific (or noise) stimulus with which the networks coevolved, we presented each network with 100 pairs of a randomly generated call from its own population versus a randomly generated heterospecific or noise stimulus.

To test the networks' preferences for local calls versus foreign conspecific calls, we presented each network with two sets of calls. In one set, networks were presented local calls versus foreign conspecific calls from one of the alternative populations, and in the second set, networks were presented local calls versus foreign conspecific calls from the second alternative population (e.g. A networks were presented A vs. AB calls in one set and A vs. AC in a second set). Thus, we generated six possible pairings of local and foreign conspecific calls. For each set we presented 100 pairs of randomly generated local calls versus randomly generated foreign conspecific calls to each of the 30 networks in each population type.

In all tests of network preference, we calculated the difference in response between the local call and the alternative call. This raw measure of discrimination is analogous to the fitness measure used during the evolution of the networks. Because the magnitude of networks' discrimination differed not only across generations but also across independently evolved replicates and populations, we generated a relative measure of preference for local calls that was comparable among pairs of stimuli, generations, replicates and populations. We generated this relative preference measure as follows. After all simulations were completed, we obtained the highest discrimination score expressed by any network at any time within that network's own replicate for the pairings of local calls versus the heterospecific calls with which they coevolved (i.e. B, C or noise). We then divided a network's raw discrimination scores for a given call pair by this maximum value for its replicate. As with our fitness measure, negative values were truncated to 0. We thereby generated a relative preference score for local calls in each pairing that varied from 0 to 1. At values close to 0, networks expressed no

discrimination. At values approaching 1, networks were expressing discrimination as strong as the highest level observed against heterospecifics (or noise) in that network's lineage. We therefore ascertained whether networks preferentially responded to local calls by comparing their average preference score in a given pair-wise test with the null expectation of 0.

Although we found that the calls evolved to be divergent among the *A*, *AB* and *AC* population types, there was variation in the call parameters that evolved among the different replicates of these population types (especially in the *AB* and *AC* populations; see Results and Figure 9.4). Such variation could result from stochasticity in the simulations or may represent alternative solutions to similar discrimination tasks. We examined how networks responded to these call variants from other replicates of their same population type and compared this to their responses toward foreign conspecific calls from other populations. By doing so, we could discern whether networks selected against foreign conspecific calls because they were from alternative population types not just alternative replicates.

To make this comparison, we generated an average call for each replicate using the mean values of all four call parameters for the given replicate. We then presented each network with the average call from its own replicate (the local call) versus each alternative replicate (the foreign replicate call) from its own population type. For example, a network from an *A* population was presented the average call for its population (the local call) and the average call of a different replicate *A* population (the foreign replicate call). Each network of the *A*, *AB* and *AC* populations was therefore presented 29 pairings of its own local call with calls from different replicates. Preferences were scored as above. From these preference scores, we generated a mean preference for local calls *within* a given population type that we then used as a null expectation against which to compare the networks' preferences for local calls versus calls from alternative population types. For example, the preference that networks from the *A* populations expressed for their own calls versus calls from the *AB* and *AC* populations was compared to the average preference that networks from *A* populations expressed for their own local calls versus those from other replicate *A* populations.

Finally, the networks might be more likely to discriminate against foreign calls as they become increasingly dissimilar from the local calls. If so, then preference for local calls should be negatively correlated with similarity between the local and foreign calls. To evaluate this possibility, we took the absolute difference between the principal component score of the average local call and the average foreign conspecific call presented to each network. We generated these values separately for both principal components. Because these data did not meet parametric assumptions, we used Spearman rank order correlation analysis to determine if the magnitude of difference between calls was associated with the average preference for local calls in a given pair type. These analyses utilised calls from across the independently evolved replicates, and so reflect patterns, if any, associated with reproductive character displacement rather than variation within a single lineage.

9.7 Results

9.7.1 How does character displacement affect preferences for conspecifics and discrimination against heterospecifics?

To answer this question, we focused only on network preferences; male calls were not allowed to coevolve. Our simulations revealed that artificial neural networks that did not encounter heterospecific calls or that interacted with different heterospecific calls diverged in their preferences for conspecific male call characters. In particular, we found that each population exerted a unique pattern of selection on the signal features that constituted conspecific advertisement calls (Figure 9.2)¹.

The eight regression coefficients measuring the networks' preference functions for the four call characters reduced to four principal components that each had an eigenvalue greater than 1.0. Together, the four principal components explained 82.3% of the variation in the regression coefficients. When we used MANOVA to compare these principal components among the three populations, we found a significant effect of population (Wilks' Lambda = 0.335, $F_{6,110} = 13.36$, $p < 0.001$). Contrasts of the populations revealed that the three populations were all significantly different from one another (A vs. AB : $F_{3,55} = 10.6$; A vs. AC : $F_{3,55} = 35.3$; AB vs. AC : $F_{3,55} = 7.7$; all contrasts are $p < 0.001$). These results indicate that the networks' preference functions for, and therefore the pattern of selection they might exert on, conspecific male traits differed among the three populations.

Although networks from the three population types differed in their preferences for conspecific calls, we found mixed evidence that sympatric and allopatric populations differed in their ability to discriminate against heterospecific calls. When we tested the networks for their preferences of conspecific versus heterospecific calls, we found they could potentially discriminate against a given heterospecific even when they had not evolved species recognition in the presence of that heterospecific species. Specifically, when given a choice of conspecific male calls versus the calls of species C , networks from the three populations differed in their ability to discriminate between conspecific and heterospecific male calls ($F_{2,57} = 83.37$, $p < 0.001$; Figure 9.3a). A Tukey–Kramer HSD test revealed that all populations were significantly different from one another at $p < 0.05$. Networks from the AC population showed the best discrimination against species C whereas networks from the A populations showed the worst discrimination against species C (Figure 9.3a). Indeed, networks from the A populations were random in their choices of conspecifics versus species C ($t_{19} = 1.5$, $p = 0.15$; Figure 9.3a). By contrast, networks from both the AB and AC populations preferred conspecific calls to those of species C (AB population: $t_{19} = 13.3$, $p < 0.001$; AC population: $t_{19} = 33.9$, $p < 0.001$; Figure 9.3a). Thus, networks from the AB population selected against heterospecific calls of species C even though they were allopatric with this species and had not evolved recognition in its presence.

¹ All of the results we present in this section (including figures) were previously published in Pfennig & Ryan (2007).

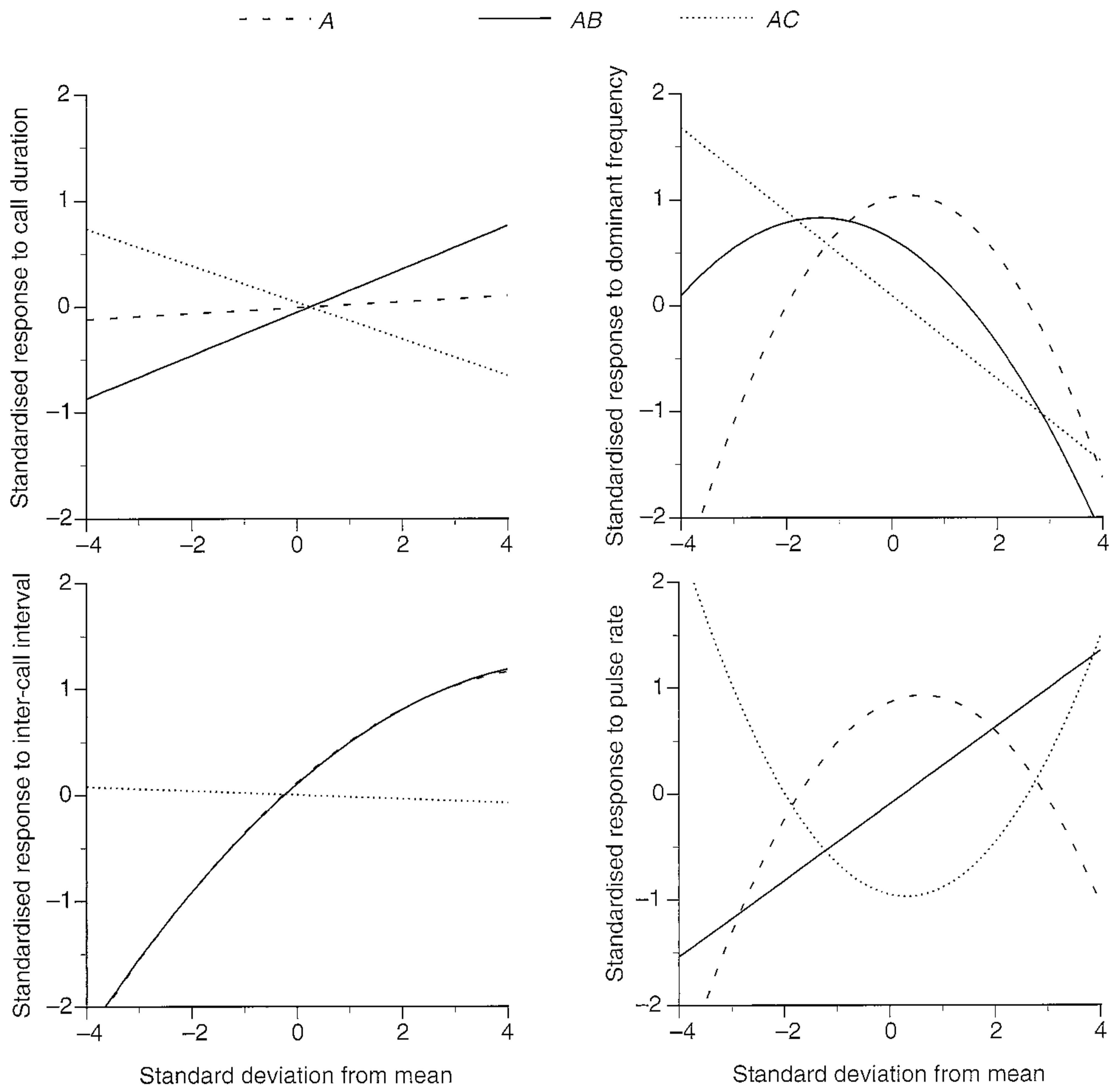


Figure 9.2. Preference functions showing the strength and nature of selection on each call character by networks of the three different population types. Preferences were measured on sets of calls in which each character was systematically varied while the other characters were held constant (see text for details). All relationships are statistically significant ($p < 0.05$), except the following: AC networks' selection on inter-call interval and call duration, A networks' selection on call duration and AB networks' selection on call duration (although for this relationship $p = 0.06$). For inter-call interval, the preference functions for A and AB overlap. Where the relationship shown is linear, the second-order regression coefficient was not significant and was dropped from the analysis.

When we presented the networks with calls from conspecifics versus those of species *B*, we found a significant difference in the populations' discrimination against heterospecifics ($F_{2,57} = 10.79$, $p < 0.001$; Figure 9.3b). A Tukey-Kramer HSD test revealed that only the AB and AC populations were significantly different at $p < 0.05$, however. The networks from the AB population were most effective at discrimination against species *B* whereas networks from the AC population were the least effective at discriminating against them

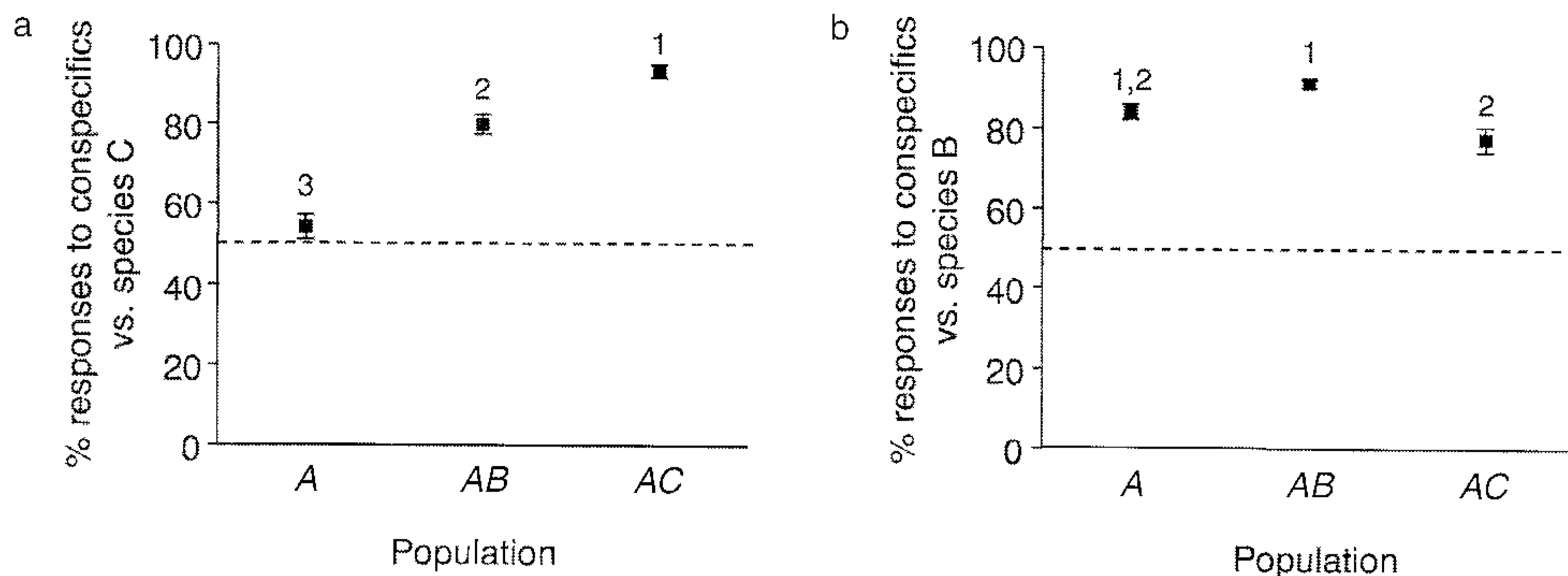


Figure 9.3. (a) Mean (\pm SE) per cent responses in which the conspecific signal was preferred in choice tests between conspecific calls of species *A* and heterospecific calls of species *C*; (b) Mean (\pm SE) per cent responses in which the conspecific signal was preferred in choice tests between conspecific calls of species *A* and heterospecific calls of species *B*. Dashed line shows 50% random expectation if networks within each population were indiscriminate in their choice of conspecifics versus heterospecifics. Within each panel, different numbers above each population mean indicate significant differences among the populations; populations that share the same number within each panel are not significantly different (see text for statistical analyses).

(Figure 9.3b). Although networks from the *A* population did not evolve conspecific recognition in the presence of species *B*, they did not differ from the *AB* networks in discrimination against species *B* (Figure 9.3b). Thus, although the networks showed divergence in their preference functions for conspecific male signals (Figure 9.2), this result did not necessarily translate into differences between sympatric and allopatric populations in discrimination of conspecific and heterospecific males. Moreover, despite differences between the *AB* and *AC* populations in their discrimination ability against species *B*, networks in all three populations significantly preferred the calls of conspecifics to those of species *B* (*A* population: $t_{19} = 23.0$, $p < 0.001$; *AB* population: $t_{19} = 36.2$, $p < 0.001$; *AC* population: $t_{19} = 8.6$, $p < 0.001$).

9.7.2 Can reproductive character displacement initiate speciation?

The divergence of signals among conspecific populations in response to heterospecifics could contribute to their reproductive isolation. We found that advertisement calls of all three populations evolved to be distinct from the ancestral call (Figure 9.4a) and from each other (Figure 9.4b). The principal component measures (PC 1 and PC 2) of the combined call parameters were both significantly different among the three populations (Wilcoxon normal approximation comparing PC 1 among population pairs: *A* vs. *AC*: $Z = -4.07$, $p < 0.0001$; *A* vs. *AB*: $Z = -6.11$, $p < 0.0001$; *AB* vs. *AC*: $Z = 5.45$, $p < 0.0001$; Wilcoxon normal approximation comparing PC 2 among population pairs: *A* vs. *AC*: $Z = -5.73$,

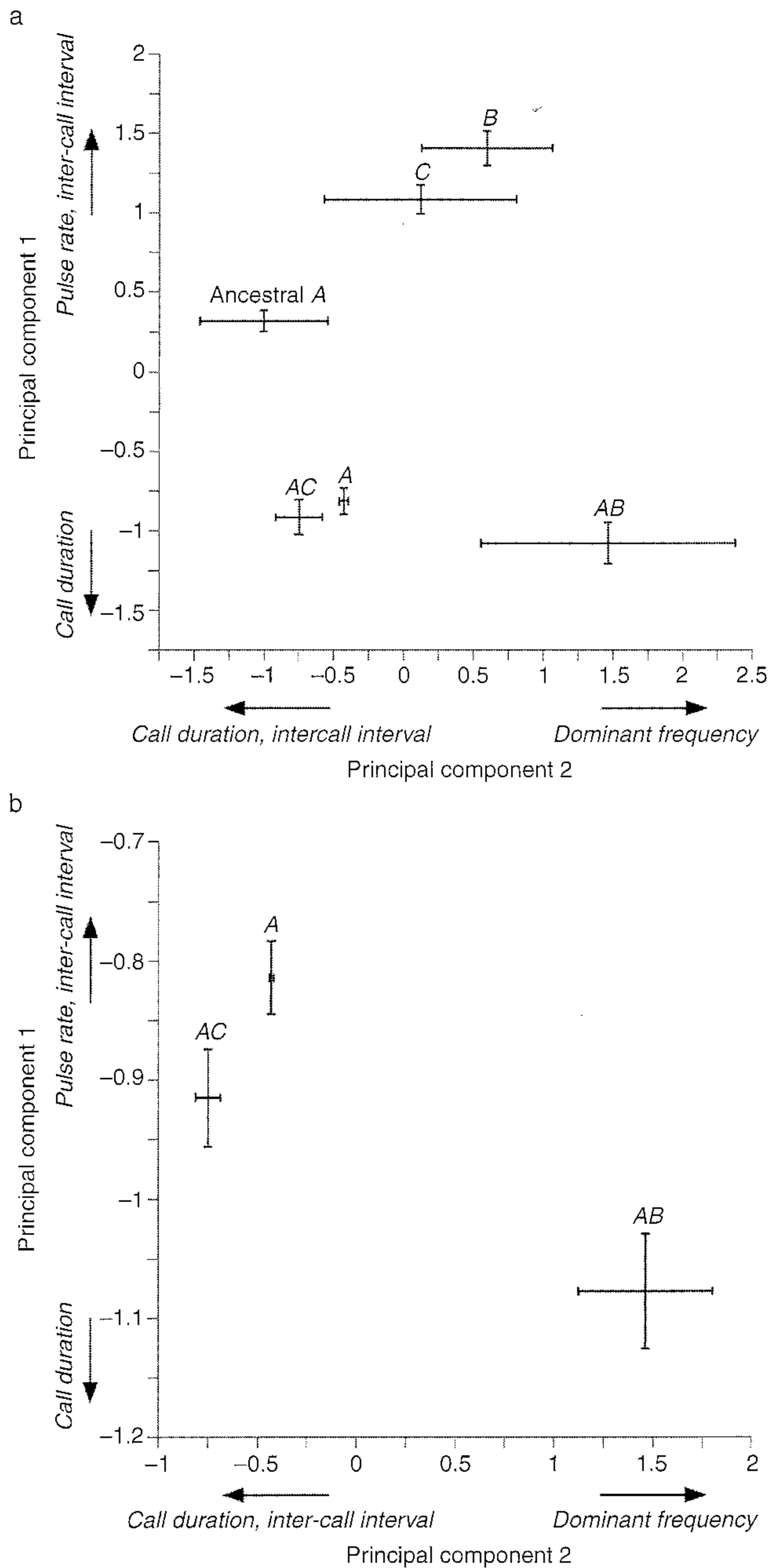


Figure 9.4. (a) Mean (\pm SD) for principal components that describe the combined variation in four call characters (dominant frequency, call duration, pulse rate and inter-call interval) for: the evolved calls from the three different conspecific populations (A, AB and AC), the heterospecific calls presented to the AB and AC populations during their evolution (B and C, respectively), and the initial conspecific call (ancestral A). Intersection of x-axis and y-axis standard deviation lines is the point of the mean for each. (b) Mean (\pm CI) for same principal components above comparing the evolved calls from A, AB and AC only. Non-overlapping confidence intervals indicate significant differences. Labels on axes indicate loading of call parameters on each principal component.

Table 9.3. Network preferences for local calls versus foreign conspecific calls. The network's population is also the population of the local call. The preference for local calls was assessed in two ways. First, mean response to local calls is compared with the null expectation of 0 if networks respond equally strongly to local and foreign conspecific calls. Second, mean response to local calls is compared with a null expectation that is the mean response to independently evolved calls from replicates of the networks' own population type. This latter contrast controlled for the possibility that networks may have been generally selective against foreign conspecific calls, not just those that diverged due to character displacement. Means and confidence intervals of preference strength for local calls from these pairings are displayed in Figure 9.5.

Network's population	Population of foreign call	t_{29} (p-value); H_0 : local call preference = 0	t_{29} (p-value), H_0 : local call preference = preference for calls from other replicates ^a
A	AB	21.48 (< 0.0001) ^b	17.96 (< 0.0001) ^b
	AC	9.46 (< 0.0001) ^b	6.70 (< 0.0001) ^b
AB	A	9.06 (< 0.0001)	3.24 (0.003)
	AC	10.72 (< 0.0001)	4.29 (0.0002)
AC	A	8.32 (< 0.0001) ^b	-1.04 (0.31) ^b
	AB	10.58 (< 0.0001)	6.71 (< 0.0001)

^a Average response to replicates by networks in A: 0.027; AB: 0.310; AC: 0.203.

^b Analysis used transformed data to meet parametric assumptions.

$p < 0.0001$; A vs. AB: $Z = 5.34$, $p < 0.0001$; AB vs. AC: $Z = -5.79$, $p < 0.0001$; $N = 30$ for each population).

We used two measures to determine if the networks preferred local calls versus foreign conspecific calls. First, we compared mean preference for local calls with the null expectation of 0 (see methods described above). Networks from the three populations significantly preferred local calls to foreign conspecific calls (Table 9.3; Figure 9.5).

Divergence of populations could potentially result from stochastic variation in preferences and calls among independently evolved lineages rather than due to character displacement per se. To control for this possibility, we also compared networks' mean preferences for local calls when presented with foreign conspecific calls with their mean preference for local calls when presented with independently evolved calls from alternative replicates of their own population type (see methods described above). We found that the A networks showed a weak preference for local calls versus calls from alternative replicates, whereas the AB and AC networks displayed relatively stronger preference for calls that evolved in their own replicate (Figure 9.5).

Although the networks discriminated against calls from alternative replicates of their own population type, they generally showed even stronger preferences for local calls when they were paired with foreign conspecific calls. In all but one pairing (AC networks presented with AC vs. A calls), the networks discriminated against foreign conspecific

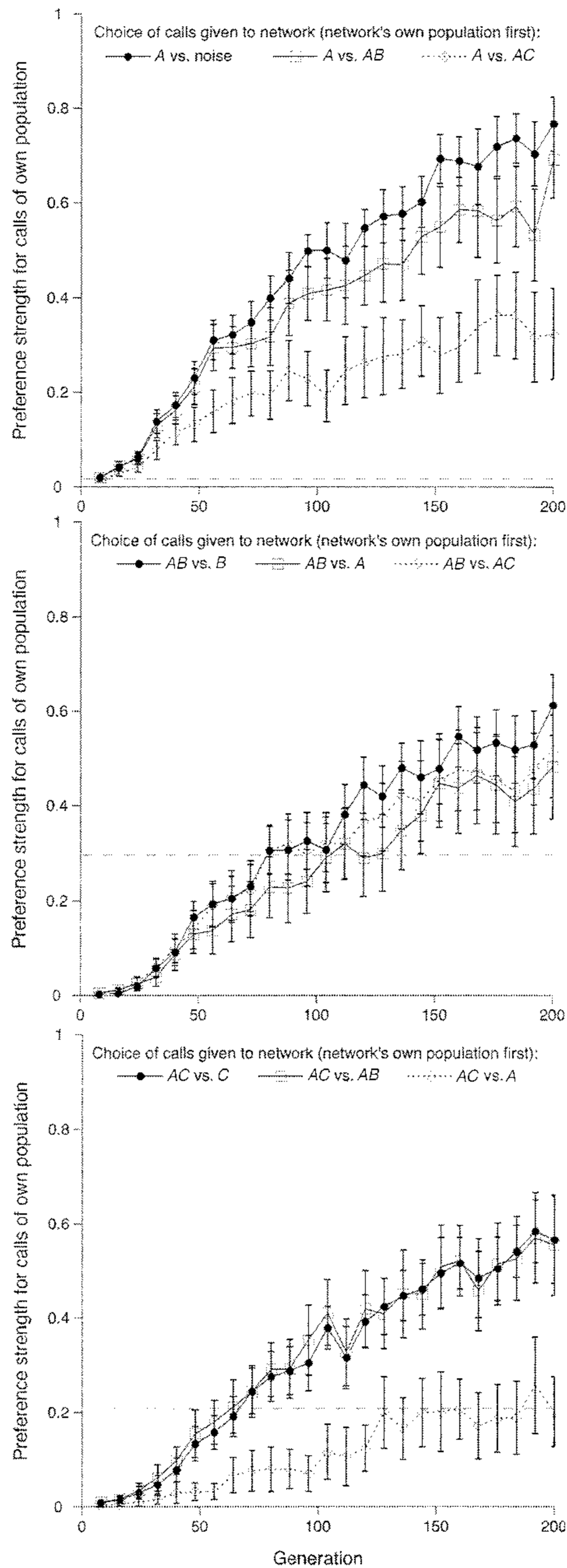


Figure 9.5. Mean (\pm 95% CI) preference for advertisement calls from networks' own population (local calls) versus calls of alternative populations (foreign conspecific calls) over time. Top panel shows results for A networks; middle panel shows results for the AB networks; and the bottom panel shows results for AC networks. Non-overlapping confidence intervals indicate significant differences. Dashed horizontal line shows null expectation derived from network preferences for local calls versus those from alternative replicates of same population type in final generation.

Table 9.4. Correlation of preference strength for local calls with the absolute differences in principal component scores of local versus foreign conspecific calls (a measure of the similarity between the calls). Spearman rank order correlation coefficient reported because data did not meet parametric assumptions. The network's population is also the population of the local call. $N = 30$ for each correlation.

Network's population	Population of foreign call	r_s (p-value) using difference in PC 1 scores	r_s (p-value) using difference in PC 2 scores
A	AB	-0.076 (0.69)	0.195 (0.30)
	AC	0.092 (0.63)	-0.366 (0.047)
AB	A	0.060 (0.75)	0.672 (< 0.0001)
	AC	0.246 (0.19)	0.393 (0.032)
AC	A	-0.345 (0.062)	0.528 (0.003)
	AB	0.126 (0.51)	0.339 (0.067)

calls significantly more strongly than they discriminated against calls from alternative replicates (Table 9.3; Figure 9.5).

The evolutionary trajectories of these preferences for local calls suggested that they arose in conjunction with the evolution of discrimination against the heterospecific calls (or noise) with which the networks coevolved (Figure 9.5). Indeed, in all of the populations, networks in the final generation exhibited similarly strong discrimination against at least one type of foreign conspecific call as they exerted against the heterospecific calls with which they coevolved (Figure 9.5).

This was exemplified in the AB population where we found no significant differences in preference for local conspecific calls among any of the three pairings the networks were presented (AB vs. B calls, AB vs. A calls and AB vs. AC calls; $F_{2,87} = 2.23$, $p = 0.11$; Figure 9.5). In the A population, we found a significant difference in preference for local calls among the three possible pairings the A networks faced ($F_{2,87} = 33.7$, $p < 0.0001$). The A networks, however, showed a similarly strong preference for local calls when they were paired with AB calls as when local calls were paired with a white noise stimulus (as revealed by a Tukey–Kramer HSD test, $p > 0.05$; Figure 9.5). The A networks showed significantly lower preference for local calls in the pairing of A and AC calls than in the other call pairings with which they were tested (Tukey–Kramer HSD test, $p < 0.05$; Figure 9.5).

Similarly, in the AC population we found a significant difference in preference for local calls among the three possible pairings they faced ($F_{2,87} = 15.9$, $p < 0.0001$). Yet, the AC networks showed a similarly strong preference for local calls when they were paired with AB calls as when local calls were paired with heterospecific C calls (as revealed by a Tukey–Kramer HSD test, $p > 0.05$; Figure 9.5). The AC networks showed significantly lower preference for local calls in the pairing of AC and A calls than in the other call pairings with which they were tested (Tukey–Kramer HSD test, $p < 0.05$; Figure 9.5).

The above results emphasise that the networks sometimes discriminated against foreign conspecific calls as strongly as they did heterospecific (or noise) stimuli. These findings also indicate that the networks did not necessarily respond to foreign conspecific calls from different population types in the same way. In both the *A* and *AC* populations, the networks discriminated against the foreign *AB* call more strongly than they selected against each other (Figure 9.5). One explanation for this pattern is that because the *A* and *AC* calls were more similar (Figure 9.4), they were less likely to discriminate against each other than against the *AB* calls. We investigated whether the differences in how networks responded to foreign conspecific calls could be attributed to the level of similarity between the local calls and a given foreign conspecific call type. For variation described by PC1, we found no associations between preference for local calls and dissimilarity of local and foreign conspecific calls (Table 9.4). By contrast, we found that the greater the difference between local and foreign conspecific calls in PC2, the stronger the preference for local calls in four of the six pairings (Table 9.4).

9.8 Discussion

Using artificial neural network models, we simulated the evolution of conspecific recognition in the presence of different heterospecifics. We found that selection to avoid mating with heterospecifics can generate divergent mate preferences for aspects of conspecific signals among different conspecific populations. Moreover, these divergent preferences can ultimately promote diversification of mating signals among conspecific populations. Critically, we found that this divergence in preferences and signals can promote reproductive isolation among conspecific populations that differ in the nature of heterospecific interactions they experience. Thus, reproductive character displacement can lead not only to enhanced differentiation of mating behaviour between species, it can also potentially initiate speciation events among populations that vary in the heterospecifics they encounter.

Many studies of reproductive character displacement assay whether females that are sympatric with a particular species of heterospecific are more likely to reject heterospecific mates than are allopatric females (reviewed in Howard, 1993). Similarly, such population differences in the ability to discriminate conspecifics from heterospecifics has been viewed as a critical prediction of reinforcement – the hypothesis that natural selection against hybridisation will promote divergent mating behaviours between hybridising species within sympatry but not allopatry (Howard, 1993; Noor, 1995; Servedio & Noor, 2003; Coyne & Orr, 2004). Yet, empirical studies are mixed as to whether they find support for this pattern (reviewed in Howard, 1993). The results of our model suggest that character displacement in female preferences for conspecific male calls does not necessarily result in differences between sympatric and allopatric populations in the ability to discriminate against heterospecifics. Moreover, because our results benefited from large sample sizes that would be unrealistic in many natural systems, such

differences as those we did observe would be difficult to detect empirically. Thus, failure to find differences in the ability to discriminate against heterospecifics between populations of sympatry and allopatry in a natural system should not necessarily result in rejection of the hypothesis that reproductive character displacement or reinforcement has occurred. Instead, reproductive character displacement might best be detected by observing differences between sympatry and allopatry in mate preferences for aspects of conspecific signals rather than by searching for differences in discrimination against heterospecifics.

Our results indicate that whether females discriminate against heterospecifics in sympatry or allopatry with a given heterospecific likely depends on female preferences for aspects of conspecific male traits and the trait distribution of the heterospecific signals relative to those preferences. For example, preference for signals with lower dominant frequency by *AC* networks would result in them discriminating against not only species *C*, the heterospecific with which they coevolved, but also species *B*, with which they had no interactions during their evolutionary history (contrast Figure 9.2 with Figure 9.4a). Thus, females' risk of mating with heterospecifics may strongly depend on the nature of female preferences for conspecifics rather than whether the females occur in sympatry or allopatry per se (Ryan *et al.*, 2003; Rodriguez *et al.*, 2004).

Not all preferences that evolve necessarily contribute to successful species recognition, however. In the case of pulse rate, for example, the preferences for higher pulse rate expressed by both the *AB* and *AC* networks could possibly put them at risk of mating with heterospecifics if this trait were important to mate choice. Females in natural systems do not weight all traits equally (Gerhardt, 1994), and the fact that both the *AB* and *AC* networks strongly discriminated against both heterospecifics (Figure 9.3) suggests that pulse rate was not heavily weighted by the networks in their responses to the male calls. Why the networks evolved the preferences for pulse rate that they did remains unclear. One explanation is that the evolution of preferences for other characters could have had a pleiotropic effect on the evolution of pulse rate preferences. The degree to which heterospecific interactions generate diversity in mate preferences through pleiotropic effects rather than due to direct selection on traits that enhance discrimination remains an open question.

That the nature of heterospecific interactions alters preferences for conspecific signals has implications beyond the effects on species recognition. Our findings indicate that divergent preferences, in turn, drive the diversification of male signals among populations. This coevolution of female preferences and male signals thereby promotes assortative mating within – and reproductive isolation among – conspecific populations.

Although we observed divergence in male signals, call evolution was not strictly caused by differences in heterospecific interactions among the different populations. All three populations diverged dramatically from the ancestral call type (Figure 9.4a). Such evolution may have occurred if, for example, certain call characters were more easily discriminated against the noisy background that all three populations experienced. Yet, despite their

similar evolution relative to the ancestral calls, the calls of the different populations also diverged from one another (Figure 9.4b). Interestingly, the calls that evolved in the populations that discriminated against heterospecifics were more variable (especially in *AB*) than those in the allopatric population, *A* (Figure 9.4). Why this was so is unclear. One explanation is that there were few optimal calls for discriminating against noise alone, but many alternative call solutions for discriminating against a given heterospecific. Generally, such variation could further promote diversification among populations.

Perhaps most critically, we found that networks preferred calls of their own population to those from alternative conspecific populations (Table 9.3; Figure 9.5). Indeed, in some cases, the networks discriminated against foreign conspecific calls and heterospecific calls similarly. These results suggest that character displacement in mating behaviours such as male signals (arising from selection to avoid heterospecifics in sympatry but not allopatry) can simultaneously promote assortative mating within sympatric and allopatric conspecific populations. In a natural system, this pattern of mate choice could generate reproductive isolation, and ultimately initiate speciation, among conspecific populations (e.g. Hoskin *et al.*, 2005).

Although networks from all three populations in our study tended to prefer local calls, networks from a given population did not necessarily show the same level of discrimination against different types of foreign conspecific calls (Figure 9.5). Such a finding indicates that the evolution of discrimination against heterospecifics does not necessarily result in the rejection (or equal treatment) of all foreign calls. Discrimination against foreign conspecific calls tended to be weaker when local and foreign conspecific calls were more similar (Table 9.4). Indeed, calls from the *A* and *AC* populations were the most similar (Figure 9.5) and networks from both populations were less discriminating against calls from the alternate population than they were against calls from the *AB* population. Similarly, when tested for their preferences of local calls versus calls from alternative replicates of their same population type, the preference for local calls was weakest in the *A* population (Figure 9.5; see also Table 9.3), which exhibited very low variation in calls across replicates (i.e. local calls and foreign conspecific calls were all similar; Figure 9.4). By contrast, the preference for local calls versus calls from alternative replicates was highest in the *AB* population (Figure 9.5; see also Table 9.3), which exhibited higher variation in calls across replicates (Figure 9.4). These results suggest that different types of heterospecific interactions may more likely contribute to reproductive isolation if they promote the evolution of opposing signal characters among conspecific populations. Thus, the particular mating behaviours that evolve in response to heterospecifics may determine whether populations become reproductively isolated.

One feature of our simulations that undoubtedly promoted the diversification of mating behaviours among the conspecific populations was the close coevolution between signals and receivers. Such a pattern of coevolution often occurs between males and females (Andersson, 1994). If, however, signal evolution (or receiver perception) is under direct countervailing selective pressures (e.g. from predators or energetic or physiological limitations) or affected indirectly by the evolution of correlated characters, divergence

among populations may in turn be limited. Predicting the circumstances under which reproductive character displacement may promote the evolutionary diversification of mating behaviours, and possibly speciation, among conspecific populations may therefore require a comprehensive understanding of the selective and correlated factors that determine the evolution of mating behaviours within and among populations.

One factor not included in our model that can dramatically affect the degree to which populations diverge is gene flow. In our model, the populations were evolving in isolation, which facilitated their divergence. Gene flow among populations can reduce the likelihood of divergence, however, by introducing trait and preference alleles from one population into others (Barton & Hewitt, 1989; Kelly & Noor, 1996; Servedio & Kirkpatrick, 1997; Barton, 2001). If migration rates are sufficiently high and if alleles introduced via gene flow spread in a population, differences among conspecific populations for mating behaviours could disappear. Yet, although gene flow typically reduces divergence, it need not eliminate divergence especially if selection is strong (Liou & Price, 1994; Kelly & Noor, 1996; Kirkpatrick & Servedio, 1999). Moreover, our findings suggest that once populations begin to diverge in mating behaviours, migrant males or females would be at a selective disadvantage because they would be less likely to mate than resident individuals (Table 9.3; Figure 9.5). Consequently, as long as gene flow does not eliminate initial differentiation of mating behaviours among populations, their divergence could counteract the effects of gene flow and thereby further enhance the likelihood that populations become reproductively isolated.

Reproductive character displacement is generally viewed as a result of reinforcement and the final stages of speciation (Dobzhansky, 1940; Howard, 1993; Coyne & Orr, 2004) or a consequence of interactions that accentuate existing species boundaries (Butlin, 1987). Our results suggest that reproductive character displacement can also initiate speciation. Such a process has been described, for example, in the green-eyed tree-frog, *Litoria genimaculata* (Hoskin *et al.*, 2005). Because most species co-occur with heterospecifics and may even occur with different heterospecifics in different parts of their range, these results further suggest that reproductive character displacement could potentially initiate 'speciation cascades' – multiple speciation events across a given species' range. Yet, whether reproductive character displacement generates diversity in this way remains an open question. Discovering the role that reproductive interactions plays between species in rapid evolutionary diversification is therefore potentially critical for assessing how mate choice contributes to the speciation process.

9.9 Conclusions

Neural network models offer a valuable tool for examining how mating behaviours may diverge between conspecific populations experiencing unique selective environments. If extrapolated to natural systems, our findings suggest that interactions with heterospecifics can cause female preferences for conspecific male characters to diverge among populations co-occurring with different species. The finding that the populations diverged in

mate preferences in response to selection to avoid heterospecific matings suggests that such interactions may facilitate the evolutionary diversification of both female mate preferences and male sexual signals. Ultimately, such a process could initiate reproductive isolation and speciation among the different populations if females from a given population fail to recognise males from different populations as acceptable mates (Howard, 1993; Hoskin *et al.*, 2005; Pfennig & Ryan, 2006). Thus, although reproductive character displacement is generally thought to occur following secondary contact of populations that already constitute two species, the process of reproductive character displacement itself could trigger further speciation events.

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