

Character displacement and the evolution of mate choice: an artificial neural network approach

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Interactions with heterospecifics can promote the evolution of divergent mating behaviours between populations that do and do not occur with heterospecifics. This process—reproductive character displacement—potentially results from selection to minimize the risk of mating with heterospecifics. We sought to determine whether heterospecific interactions lead to divergence of female preferences for aspects of conspecific male signals. We used artificial neural network models to simulate a mate recognition system in which females co-occur with different heterospecifics in different populations. Populations that evolved conspecific recognition in the presence of different heterospecifics varied in their preferences for aspects of conspecific male signals. When we tested networks for their preferences of conspecific versus heterospecific signals, however, we found that networks from allopatric populations were usually able to select against heterospecifics. We suggest that female preferences for aspects of conspecific male signals can result in a concomitant reduction in the likelihood that females will mate with heterospecifics. Consequently, even females in allopatry may discriminate against heterospecific mates depending on the nature of their preferences for conspecifics. Such a pattern could potentially explain cases where reproductive character displacement is expected, but not observed.

Keywords: reproductive character displacement; speciation; mate choice; sexual selection; population divergence; species recognition

1. INTRODUCTION

Reproductive character displacement is the process by which mating behaviours diverge between populations that do and do not occur with heterospecifics. Because hybrids often have reduced fitness relative to pure-species types, selection should favour behaviours in sympatry, but not allopatry, that enable females to identify conspecific mates (a process termed reinforcement; Dobzhansky 1940; Howard 1993; Servedio & Noor 2003; Coyne & Orr 2004). Reproductive character displacement can also arise without reinforcement, as when heterospecific signals interfere with a female's ability to discriminate among conspecific mates (Wollerman & Wiley 2002). Whether reproductive character displacement occurs has received mixed support. Many studies have found that sympatric individuals are more discriminating against heterospecifics than are allopatric individuals (reviewed in Howard 1993; e.g. Noor 1995; Saetre *et al.* 1997; Gabor & Ryan 2001). Other studies however have failed to find such a pattern (Butlin 1987; e.g. Gregory *et al.* 1998; Gray & Cade 2000).

Females can exercise mating preference within as well as among species. Indeed, the process of mate recognition is probably not sensitive to conspecifics and heterospecifics as special categories, but instead reacts

to individuals as being more or less appropriate mates (Patterson 1982; Ryan & Rand 1993; Ryan *et al.* 2003; but see Boake *et al.* 1997). Thus, selection influencing decision criteria that decrease the chances of mating with heterospecifics could concomitantly affect mate preferences for conspecifics (Ryan & Rand 1993; Pfennig 1998; Ryan & Getz 2000). Empirical studies have shown this to be the case (Gerhardt 1994; Pfennig 2000; Ryan & Getz 2000; Hankison & Morris 2003; Höbel & Gerhardt 2003). Yet such studies do not necessarily reveal whether differences in females' mate preferences for conspecific signals will result in differential ability to discriminate against heterospecifics in sympatric versus allopatric populations.

Also unclear is whether the particular preferences that evolve in a given population depend on the characteristics of the heterospecific species with which females interact. Interactions with different heterospecifics in different populations could generate divergent mate preferences among conspecific populations that co-occur with different species (Howard 1993). Yet whether or not female preferences evolve similarly in response to different heterospecifics remains largely unknown.

We address these issues using 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

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(Enquist & Ghirlanda 2005). Artificial neural network models are also 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 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 recognized (Phelps & Ryan 1998, 2000; Ryan & Getz 2000; Phelps *et al.* 2001; Ryan *et al.* 2001). They can also be used to evaluate how the evolution of conspecific recognition causes diversification of advertisement signals and the potential role of this diversification in speciation (Pfennig & Ryan 2006).

In this study, we investigated how interactions with different heterospecifics affect the evolution of female preferences for conspecific signals. We further examined whether divergence in female preferences for aspects of conspecific signals resulted in differential discrimination against heterospecifics in sympatric and allopatric populations. We found that although interactions with heterospecifics generate divergent preferences for the properties of signals that females use to recognize conspecifics, this does not necessarily translate into differences between populations in their discrimination against heterospecifics.

2. MATERIAL AND METHODS

Recent work has shown that artificial neural network models can offer insights into how female mate preferences evolve (Phelps & Ryan 1998, 2000; Phelps *et al.* 2001; see also Enquist & Ghirlanda 2005). For example, 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.

In this study, we used artificial neural networks to simulate the evolution of acoustic mate recognition. We based the 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, *Spea 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. Yet empirical studies of character displacement can be confounded by clinal variation, covariation in habitat, and history, all of which could generate patterns consistent

with, but not caused by, character displacement. We therefore used elements of this system to inform a model aimed at investigating how heterospecific interactions affect the evolution of female mate preferences. 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 our modelling efforts.

Our model simulated the evolution of species recognition in three types of populations in which males call to attract females as mates. Although we simulated the evolution of species recognition for acoustic signals, our results probably can be generalized to other sensory modalities. In the first population type, networks were selected for the ability to discriminate conspecific acoustic stimuli of 'species A' from white noise. This white noise stimulus served as a control for the presence of a second stimulus and provided a means of assaying the networks' ability to recognize the conspecific signal. This population type is referred to as 'A' and mimics evolution of conspecific recognition in the absence of heterospecifics. In the second population type, networks were selected to discriminate between conspecific stimuli of 'species A' and stimuli of a heterospecific, 'species B'. This population type is referred to as 'AB'. Finally, in the third population type, networks were selected to discriminate between conspecific stimuli of 'species A' and stimuli from a second heterospecific, 'species C'. This population type is referred to as 'AC.'

(a) *The model*

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 feedback 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, 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)

$$\mathbf{a}^1(k) = \text{tansig}((\mathbf{I}\mathbf{W}^{1,1}\mathbf{p} + \mathbf{L}\mathbf{W}^{1,1}\mathbf{a}^1(k-1)) + \mathbf{b}^1), \quad (2.1)$$

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 the hidden layer; $\mathbf{LW}^{1,1}$ 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 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

$$\mathbf{a}^2(k) = \text{purelin}(\mathbf{LW}^{2,1} \mathbf{a}^1(k) + \mathbf{b}^2), \quad (2.2)$$

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. As noted above, output values were not limited. They ranged from a minimum observed value of -3670 to a maximum observed value of 3607 . For further details and schematics of the network architecture, see Demuth & Beale (1997) and Ryan & Getz (2000).

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 previously. The matrix values used to specify each network were initially uniformly randomly generated with values constrained between -1 and 1 . We then presented each network a conspecific stimulus and either a noise stimulus or one of the 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 and 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 maximize their responses to conspecifics while minimizing 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 probably operates to maximize the likelihood of choosing the correct species while minimizing 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 0.

Using these fitness measures, we selected the networks that were passed to the next 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 represented in the next generation

than did networks with lower fitness. Using this procedure, we selected 100 networks at random with replacement from those networks in the preceding generation.

Following this selection process, all networks (except a single network with the highest fitness in the previous generation) underwent mutation. 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. Previous work varying the nature of this mutation regime suggests that alterations do not appear to affect the general outcome of the simulations.

We repeated this selection and mutation process for 1000 generations, and 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.

(b) 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 synthesized 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, 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 is given in table 1.

Using the randomly chosen parameters, each call was synthesized 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 to the onset of the call (the onset of the call in the call matrix

Table 1. Mean (\pm s.d.) of call parameters for each species, measured in terms of matrix columns or rows. (See text for description of how calls were generated. The calls were presented to the networks in a 35×190 frequency by time matrix.)

call parameter	species		
	A	B	C
call duration (columns)	62.6 (7.9)	9.1 (0.7)	62.4 (5.0)
inter-call interval (columns)	72.0 (1.7)	64.8 (0.9)	87.6 (4.7)
call pulse rate (pulses/column)	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)

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 of the same size as that of the male calls. Moreover, after generating the male calls as described previously, 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 standardized, 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 1), the multivariate means of the call parameters were significantly different among all the 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 $\chi^2_4 = 131.83$, $p < 0.001$). A principal component analysis reduced the four call characters to two principal components that had eigenvalues greater than 1.0. The means (\pm s.e.) for the three species for these principal components are presented in figure 1 and show how the calls were distributed among the three species in multivariate space.

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).

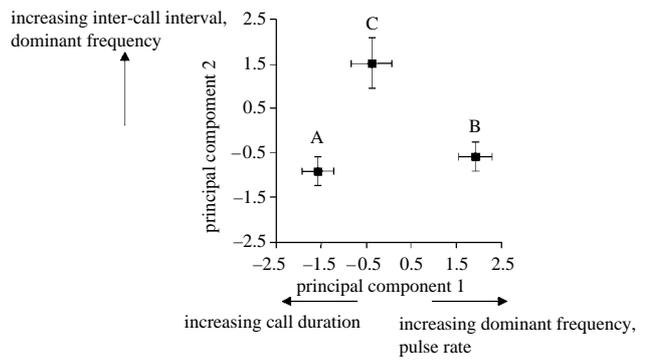


Figure 1. Means (\pm s.e.) for the first two principal components describing variation in the four call parameters used to generate the male calls for each species. Letters above the means are the species identities; $n = 20$ for all three groups.

(c) Testing and analyses of networks' responses

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 s.d. below the mean for that character to 4.0 s.d. above the mean in 0.5 s.d. 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 1). 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 standardized 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, then 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 standardized 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-order and one second-order regression coefficient for each of the 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

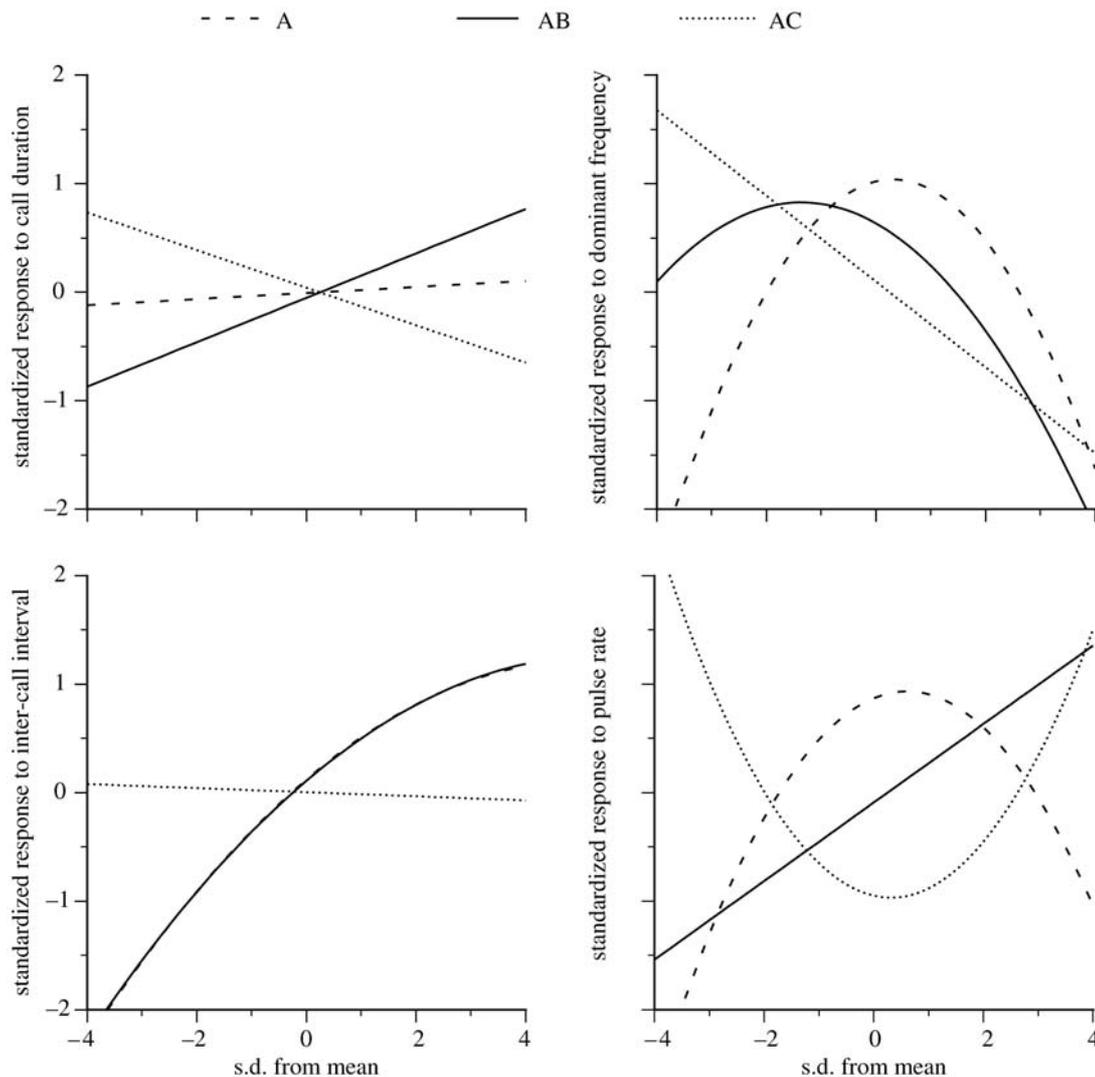


Figure 2. Preference functions showing the strength and nature of selection on each call character by networks of the three different population types. Preferences were measured on sets of calls in which each character was systematically varied while the other characters were held constant (see text 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.

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. For all analyses, the data met parametric assumptions.

3. RESULTS

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 2).

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 versus AB: $F_{3,55} = 10.6$, $p < 0.001$; A versus AC: $F_{3,55} = 35.3$, $p < 0.001$; AB versus AC: $F_{3,55} = 7.7$, $p < 0.001$). 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 that 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 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 3). Indeed, networks from the A populations were random in their choices of conspecifics versus species C ($t_{19} = 1.5$, $p = 0.15$; figure 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 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.

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 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 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 3b). Thus, although the networks showed divergence in their preference functions for conspecific

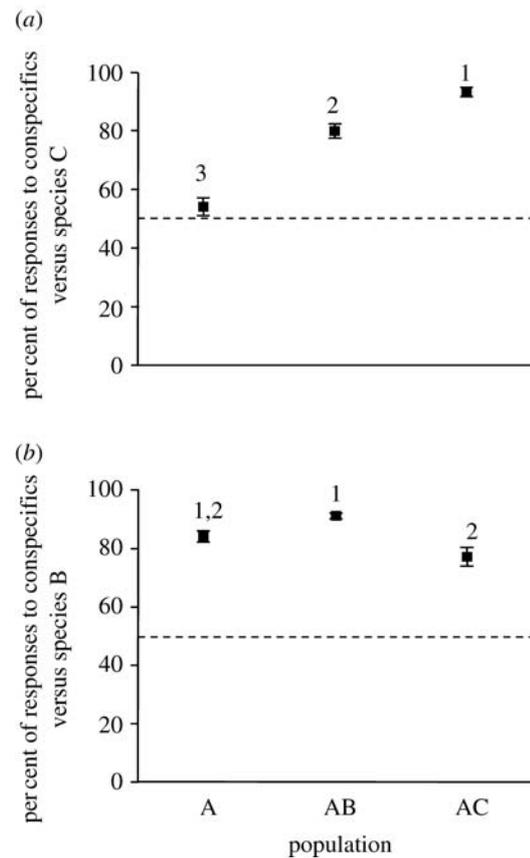


Figure 3. (a) Mean (\pm s.e.) per cent responses in which the conspecific signal was preferred in choice tests between conspecific calls of species A and heterospecific calls of species C. (b) Mean (\pm s.e.) per cent responses in which the conspecific signal was preferred in choice tests between conspecific 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).

male signals (figure 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 the 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: $t_{19} = 8.6$, $p < 0.001$).

4. DISCUSSION

Using artificial neural network models to simulate 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. Our findings are consistent with the prediction that selection to avoid interactions with heterospecifics will lead to reproductive character displacement. Perhaps more

critically, our results suggest that female preferences will not evolve in response to heterospecifics in the same way in different populations. Moreover, our findings indicate that females may discriminate against heterospecifics even if they have had no prior encounters with them.

Many studies of reproductive character displacement assay whether females sympatric with a particular species of heterospecific are more likely to reject heterospecific mates than are females from allopatry (reviewed in Howard 1993). Empirical studies are mixed as to whether they find support for this pattern (see §1). 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 has occurred between the populations.

Furthermore, reproductive character displacement as measured by 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 hybridization will promote divergent mating behaviours between hybridizing species within sympatry and, concomitantly, between populations in allopatry and sympatry within each species (Howard 1993; Noor 1995; Servedio & Noor 2003; Coyne & Orr 2004). Our results suggest that failure to support this prediction should not necessarily lead to a rejection of the reinforcement hypothesis. 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 probably depends on female preferences for aspects of conspecific male traits and the trait distribution of the heterospecific signals relative to those preferences. For example, contrasting the networks' preference functions for signal features of conspecific calls with the location that those calls occupied in acoustic space (cf. figures 1 and 2), we see that species A is at a potentially higher risk of choosing species C if presented conspecific versus heterospecific signals, but would be likely to select against species B in choice tests between this species' signals and conspecific signals. Similarly, the preferences for conspecific signals by AB networks would result in them discriminating against not only species B, the heterospecific with which they coevolved, but also species C, with which they had no interactions during their evolutionary history.

Networks from the AC population show the same result. 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).

Our findings suggest that heterospecific interactions may alter the nature of mate preferences for conspecific signals. In turn, these interactions may dictate not only whether, but also how well, conspecific signals are discriminated from other heterospecific signals. Although the networks from the AB and AC populations discriminated against both heterospecific species (B and C), networks in each population discriminated against the heterospecific with which they evolved more strongly than they discriminated against the heterospecific to which they had not been exposed (figure 3). This result underscores the possibility that in natural systems heterospecifics will not be treated equivalently (e.g. Rodriguez *et al.* 2004). Such a pattern may result if the preferences for conspecific signals that evolve to facilitate discrimination against a focal heterospecific species reduce the ability to discriminate against a novel heterospecific species.

Moreover, not all preferences that evolve necessarily contribute to successful species recognition. In the case of pulse rate, for example, the preferences for higher pulse rate expressed by both the AB and the AC networks would possibly put them at risk of mating with heterospecifics if this trait were important to mate choice (contrast table 1 with figure 3). 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 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.

Our finding that the populations diverged in mate preferences suggests that heterospecifics can promote different patterns of sexual selection among conspecific populations. One factor not included in our model that could potentially override divergence among the populations, however, is gene flow among the populations. By not including gene flow, our model was essentially an allopatric model in which the populations evolved divergent mate preferences in isolation of one another. Gene flow is typically thought to counteract divergent selection between populations, because alleles are continuously dispersed from one population to another thereby preventing genetic divergence (Barton & Hewitt 1989; Kelly & Noor 1996; Servedio & Kirkpatrick 1997; Barton 2001). Yet, despite moderate gene flow, populations may still diverge provided that selection is strong enough to override the effects of gene flow, which would tend to

homogenize the behaviours of the disparate populations (Liou & Price 1994; Michalak *et al.* 2001). Moreover, as populations diverge in mating behaviours, dispersing individuals may have lower mating success because they possess inappropriate preferences or mating signals in their new population. Thus, provided that gene flow does not preclude the evolution of initial differentiation of mating behaviours, divergence of mating behaviours among populations may concomitantly select against dispersal behaviours.

Another element not explicitly considered in the model is that advertisement signals and mate preferences used in species recognition could be learned. Learning can affect the expression of preferences or advertisement signals either because individuals alter their mating behaviour in response to heterospecifics or because the way in which mating behaviours are learned evolves in response to heterospecifics. Although our model simulated the evolutionary adjustment of preference in response to heterospecifics, the model could potentially be generalized to apply to the optimization of species recognition by learned adjustments in preference.

5. 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. Indeed, when diverging female preferences exert a unique pattern of selection on different aspects of male calls in different populations (as was observed here; figure 2), male calls should diverge concomitantly (Pfennig & Ryan 2006). Ultimately, such a process could initiate reproductive isolation and speciation among the different populations, if females from a given population fail to recognize males from different populations as acceptable mates (Howard 1993; Hoskin *et al.* 2005; Pfennig & Ryan 2006). Thus, while reproductive character displacement may result from speciation processes such as reinforcement, the process of reproductive character displacement itself could potentially initiate speciation events.

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