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No preference in female sika deer for conspecific over heterospecific male sexual calls in a mate choice context

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Keywords

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Abstract

Mating signals can be used both in contexts of species recognition and mate quality assessment. This study examines species recognition abilities in oestrous females presented with male mating calls from both conspecifics and closely related allopatric heterospecifics. Red deer and sika deer are naturally allopatric polygynous species capable of hybridization during sympatry. Male mating calls are sexually selected and differ greatly between species. Previous work indicated that most but not all oestrous red deer hinds prefer male mating calls from conspecifics over heterospecific sika deer. Using two-speaker playback experiments, we extend this examination by measuring the preference responses of oestrous sika deer hinds to these stimuli. We predicted that oestrous sika deer hinds will show little flexibility in behavioural responses and prefer conspecific calls over heterospecific calls, similar to those of red deer hinds. In contrast, sika deer hinds showed high levels of flexibility and no difference in overall preference behaviours, suggesting that vocal behaviour does not provide a solid barrier to hybridization in this species. The asymmetry in heterospecific preference responses between these species is discussed in relation to possible causation and hybridization patterns observed in free-ranging populations.

Introduction

Mate choice decisions based on mating signals play a key role in speciation, sexual selection and signal evolution (Ryan & Rand, 1993a; Pfennig, 1998; Panhuis *et al.*, 2001). Within species, mating signals can be used by reproductive rivals to advertise and assess competitive ability (within-sex mate competition) and by potential mates to broadcast and evaluate mate quality (between-sex mate choice) in an effort to increase reproductive success (Andersson, 1994). These signals can also promote speciation by functioning as a pre-zygotic reproductive isolation mechanism between species (Dobzhansky, 1937; Mayr, 1963), for example, individuals may use mating calls as an early filter to separate appropriate mates (conspecifics) from inappropriate mates (non-conspecifics) (Ord, King & Young, 2011). Females should generally be even more cautious, or less flexible, than males when determining species identity as females usually have higher costs associated with mismatched matings (Trivers, 1972; Andersson, 1994). Although it is predicted that species will respond more strongly to mating signals from conspecifics than from heterospecifics, deviations from this pattern are not uncommon as species can either show no difference in responses between signals or show stronger responses to heterospecific calls (Ord & Stamps, 2009; Ord *et al.*, 2011). When individuals do not discriminate in favour of conspecific mating signals,

hybridization is a possible outcome if the species are reproductively compatible, although hybridization does not necessarily carry fitness consequences (Pfennig, 2007).

Flexibility in responses to heterospecific mating signals can be influenced by a number of factors, such as familiarity (allopatric vs. sympatric) and phylogenetic distance between the species, scarcity of conspecific mates, the sex or reproductive state of the receiver, signal reliability, sensory biases, perception abilities, the degree of overlap with conspecific signals, and hybrid fitness (Ryan & Rand, 1993a,b; Endler & Basolo, 1998; Noor, 1999; Veen *et al.*, 2001; Pfennig, 2007; Svensson *et al.*, 2007; Wellenreuther, Tynkynen & Svensson, 2009; see Wirtz, 1999; Ord *et al.*, 2011 for review). As such, asymmetries in discrimination abilities or preference responses to mating signals between hybridizing species can occur (e.g. Derégnaucourt & Guyomarc'h, 2003), which may strongly influence the direction and speed of hybridization and introgression.

When females closely evaluate male mating signals during recognition decisions involving both species and mate quality, potential recognition errors can occur, especially if heterospecific signals resemble signals from high-quality conspecific males (Ryan & Rand, 1993a; Pfennig, 1998). In locations where closely related species are sympatric, females may constrain their directional preference for conspecific male traits to guard against potential heterospecific matings

(Pfennig, 2000; Pryke & Andersson, 2008). In contrast, females from allopatric species may continue to prefer signals that indicate high-quality conspecifics as the risk of hybridization is low.

Most studies examining species discrimination of acoustic cues have focused on insects (Doherty & Howard, 1996; Izzo & Gray, 2011), anurans (Gerhardt, 1994; Ryan & Rand, 1999) and birds (Searcy, 1990; Derégnaucourt & Guyomarc'h, 2003). Although a few mammalian studies have examined acoustic species discrimination by males (primates, Mitani, 1987; Muroyama & Thierry, 1998; bats, Schuchmann & Siemers, 2010) or females of unknown reproductive state (primates, Muroyama & Thierry, 1998), even fewer have examined species discrimination in oestrous females (mice, Meindl, 2009; deer, Wyman *et al.*, 2011). Given complex cognition within mammals, extending recognition studies to this taxon would be highly beneficial to the study of speciation and sexual selection. Here, we examine species recognition abilities in oestrous females presented with sexually selected, but acoustically dissimilar, male mating calls from both conspecifics and closely related allopatric heterospecifics. In such cases, females are likely to reject dissimilar heterospecific signals as coming from potential mates due to their preference for specific traits linked to conspecific male quality, unless heterospecific traits resemble exaggerations of preferred conspecific traits.

Scottish red deer *Cervus elaphus scoticus* and Japanese sika deer *Ce. nippon nippon* are closely related polygynous species that are naturally allopatric, but do hybridize when populations become sympatric (see species details in Materials and methods section). During the reproductive season, both species produce loud and prominent, but acoustically dissimilar, male mating calls, which are thought to be sexually selected. Previous playback experiments found that most oestrous red deer hinds preferred conspecific mating calls over heterospecific sika deer mating calls, although there were individual differences in responses, with some hinds directing more preference behaviours towards heterospecific than conspecific calls (Wyman *et al.*, 2011). In this study, we contrast these previous results with similar two-speaker playback experiments designed to examine the behavioural reactions (preference and attention) of oestrous sika deer hinds to paired presentations of conspecific versus heterospecific male mating calls. As the focal hinds in both experiments had no previous experience with the other species, these studies replicate situations of first contact between two populations of naturally allopatric species that become locally sympatric due to introductions or distribution shifts. Given the highly dissimilar acoustic structure between these two types of sexually selected mating calls, we predict that oestrous sika deer hinds will show little flexibility in behavioural responses to these call types and prefer conspecific over heterospecific calls, similar to results seen in oestrous red deer hinds. As such, reproductive responses to heterospecific calls would function as a potentially strong reproductive isolating mechanism between these species to prevent hybridization. Additionally, we will also examine attention responses to both stimuli types and will discuss possible causes for any detected asymmetries in response behaviours between sika deer and red deer.

Materials and methods

Species description and study location

In polygynous red deer and sika deer, males compete with each other for access to female harems or territories where females congregate (Clutton-Brock & Albon, 1979; Miura, 1984). The male mating calls of these species are thought to be sexually selected within contexts of male competition and female choice (McComb, 1991; Minami & Kawamichi, 1992; Reby *et al.*, 2005, 2010; Charlton, Reby & McComb, 2007). Although these calls may have similar functions, their acoustic properties are quite different. Male red deer produce low-pitched, often harsh sounding 'roars' (fundamental frequency (F0) mean = 106.9 Hz, range = 61.7–136.8 Hz; Reby & McComb, 2003) in multi-call bouts, whereas male sika deer predominantly generate very tonal high-pitched single 'moans' with a distinctive single rise and fall in F0 (F0 range = 196–1187 Hz; Minami & Kawamichi, 1992; Fig. 1). Despite additional large phenotypic differences in size, coloration and antler configuration (e.g. red deer are about twice the size of sika deer; Geist, 1998), these species are closely related (Ludt *et al.*, 2004) and have the ability to interbreed and produce fertile hybrids in either direction (sika deer stag × red deer hind or vice versa) (Senn & Pemberton, 2009). Although naturally allopatric, introductions of Asian sika deer into Europe since the 19th century have resulted in localized hybridization and introgression in free-living populations (Abernethy, 1994; Bartoš, 2009; Senn & Pemberton, 2009).

Playback experiments were conducted with 15 sika deer hinds (age: 2–7 years old) on 24–25 November 2010 at La Haute Touche, Obterre, France. Playback stimuli included paired presentations of male mating calls from conspecific Japanese sika deer versus heterospecific Scottish red deer. No focal hinds used in this study had previous direct contact with red deer. All experiments were conducted in accordance with the Association for the Study of Animal Behaviour/Animal Behaviour Society guidelines for the ethical use of animals in

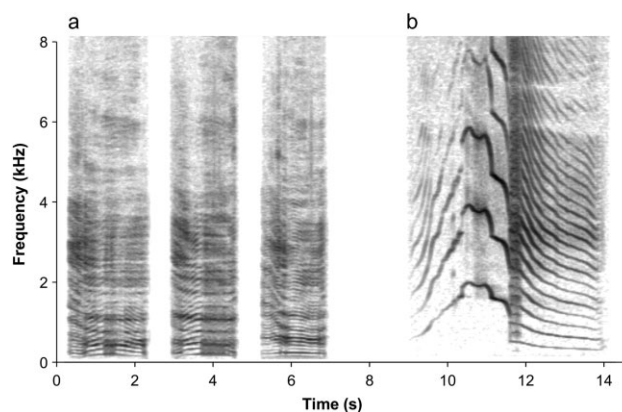


Figure 1 Spectrogram of adult male mating vocalizations: (a) bout of three red deer roars and (b) a sika deer moan.

Table 1 Acoustic profiles of deer exemplars used in playback experiments (mean \pm SD; range)

Species exemplar	Call duration (s)	Mean F0 (Hz)	Min F0 (Hz)	Max F0 (Hz)	Absolute slope (Hz s ⁻¹)
Red deer	1.8 \pm 0.4; 1.3–2.7	116.7 \pm 19.9; 76.9–160.3	72.3 \pm 20.3; 40.1–122.1	142.4 \pm 23.0; 84.4–190.4	81.7 \pm 33.8; 30.1–187.3
Sika deer	4.8 \pm 1.0; 3.0–7.1	1045.5 \pm 147.6; 753.9–1335.8	244.1 \pm 34.0; 183.0–357.5	2094.6 \pm 297.3; 1477.0–2621.7	715.1 \pm 136.0; 553.4–926.0

SD, standard deviation.

research, and with the procedural and ethical authorization of the French Ministry of Agriculture (authorization number A37801).

Oestrus synchronization

In order to conduct experiments during periods of peak oestrus, hind oestrous cycles were synchronized prior to playback trials. Intra-vaginal sponges (1 \times 45 mg; Intervet Shering-Plough Animal Health, Angers, France), containing fluorogestone acetate, provided a steady, continuous release of progesterone that inhibited normal hormone cycling by preventing follicular growth and the subsequent release of oestradiol. Intramuscular injections of cloprostenol (75 μ g; Estrumate, Intervet Shering-Plough Animal Health, Beaucauzé, France) were administered 8 days after sponge insertion. Sponges were removed 12 days post-insertion, and oestrus and ovulation were induced with subsequent intramuscular injections of equine chorionic gonadotropin pregnant mare serum gonadotropin (PMSG; 100 UI). Playback experiments were conducted 35–48 h after sponge removal and PMSG injection, during the predicted window of peak oestrus (see Reby *et al.*, 2010).

Playback stimuli

Male sexual calls from four Japanese sika deer and four Scottish red deer were used as acoustic stimuli in playback experiments. The type of sexual calls selected for each exemplar (sika deer ‘moans’ and red deer ‘roars’) are the most predominant call types emitted by males during the reproductive season (Clutton-Brock & Albon, 1979; Minami & Kawamichi, 1992) and are thought to be used in male competition and female choice (Minami, 1997; Reby *et al.*, 2005; Charlton *et al.*, 2007). All males were unfamiliar to the focal females and were sampled from populations that contain little to no known history of previous hybridization events (R. Harrington & M. Verdier, pers. comm.). Red deer roars were recorded at the Redon Experimental Farm, Clermont-Ferrand, France, in 1996 and sika deer moans were recorded at a private farm in Waterford, Ireland, in 2007 (see Wyman *et al.*, 2011 for recording specifications). The acoustic properties of male mating calls from these populations (Table 1) were similar to those described in other populations. The following acoustic parameters were measured using Praat version 5.1.13 (Boersma & Weenink, 2009): duration (s), F0 (Hz) and absolute slope of F0 (Hz s⁻¹). Calls were converted to pitch objects (‘Perio-

dicity: To pitch’ command, time step = 0.01 s, pitch floor/ceiling = 100/2700 Hz for sika deer, 30/180 Hz for red deer), and the pitch contour was adjusted as needed within the PitchEditor window in the Praat software programme.

Male calls were arranged into six bouts of similar duration with the number of calls per bout based on the naturally observed calling behaviour for each species. Sika deer bouts consisted of one call per bout (six total unique calls per individual), whereas red deer bouts contained one to four calls per bout (16 total calls per individual consisting of 8–11 unique calls, with 0.5 s between calls within bouts, and no calls used more than twice or repeated within bouts). Average bout duration and total acoustic duration per playback were similar between species exemplars (see Wyman *et al.*, 2011). All playback stimuli had a sampling frequency of 44.1 kHz, and bouts were normalized to 98% of maximum intensity using Cool Edit Pro 2.0 (Syntrillium, Phoenix, AZ, USA). During each playback, paired sequences of red deer and sika deer calls were broadcast from two speakers (one exemplar per speaker). Six bouts from each exemplar were organized into matched pairs with 2 s between bouts within a pair and 20 s between bout pairs, and the leading call in each bout pair alternated between species (see Charlton *et al.*, 2007; Wyman *et al.*, 2011). A Latin square design was used to randomize the playback pairings, the leading call within each trial and the speaker location of each exemplar. Overall, the total duration of each playback trial was approximately 3 min.

Playback protocol

Playback experiments were conducted in a partially wooded rectangular enclosure (675 m²), which hinds had previous access to as a feeding location. Two Anchor Liberty 6000 HIC amplified speakers (Anchor Audio, Inc., Carlsbad, CA, USA) were concealed behind camouflage netting against the far wall of the arena, 1 m above the ground and 14 m apart. Proximity zones, which extended 8 m out from the front of each speaker, were demarcated with naturally occurring sticks. Playback sequences were played through a Toshiba NB205 netbook computer (Toshiba America, Inc., New York, NY, USA) connected to the speakers with coaxial cables. Playback experiments were conducted on one hind per trial. Trials commenced after the focal hind was calm and located near a feeding station at the centre of the enclosure, equidistant from the two speakers. Acoustic stimuli were broadcast at sound pressure levels of 95 decibels at 1 m from the speaker, as measured by a CEM DT-805 sound

Table 2 Behavioural responses by sika deer hinds to conspecific versus heterospecific male mating calls

Behavioural measure		Conspecific stimuli (range, median, IQR ^a)	Heterospecific stimuli (range, median, IQR ^a)	Wilcoxon test (z-score, P-value)
Entering proximity zones	Instances	0–6.00 1.00, 3.50	0–6.00 2.00, 3.50	$Z_{15} = -0.63$ $P = 0.527$
	Total time (s)	0–91.12 2.55, 51.11	0–105.14 13.34, 44.18	$Z_{15} = -0.27$ $P = 0.79$
Looking at speakers	Instances	2.00–25.00 7.00, 8.00	3.00–15.00 6.00, 4.00	$Z_{15} = -0.74$ $P = 0.461$
	Total time (s)	3.35–51.19 8.67, 13.83	3.75–27.32 8.85, 7.69	$Z_{15} = -0.51$ $P = 0.609$

^aIQR, interquartile range of behavioural responses (range of the middle 50% of behavioural scores) calculated as the difference between the third quartile and first quartile.

level meter (Shenzhen Everbest Machinery Industry Co., LTD, Nanshan, Shenzhen, China) with C-weighting. Focal hind behavioural responses were video recorded using a Sony Handycam MiniDV HC52 camcorder (Sony Corporation of America IR, New York, NY, USA) from a hidden, elevated position.

Behaviour analysis

'Preference' and 'attention' behaviours were monitored in hinds in response to playback stimuli from the start of the playback until 2 min post-playback. Behaviours were coded from video recordings using digital video analysis software Gamebreaker 7.0.121 (Sportstec, Sydney, Australia) at 25 frames per second. 'Preference' was measured as the number of instances of entering the proximity zone and the total time spent within the proximity zone in front of each speaker, marked when the first leg of the focal hind crossed the zone demarcation line. 'Attention' was measured as the number and total duration of looks directed towards each speaker by stationary hinds or hinds that stopped two steps after the initiation of a look. Behavioural coding was carried out by MTW using a methodology identical to Wyman *et al.* (2011), a method that produced highly reliable results in a double-coding exercise (98.3% agreement between two coders for trials with non-zero values). The operational measures of preference behaviours were based on previous studies examining intraspecific mate choice decisions in female red deer (Charlton *et al.*, 2007; Reby *et al.*, 2010).

The distribution of behavioural responses was visualized with box plots, using whisker limits set at $1.5 \times$ interquartile range (IQR) of the lower and upper quartile. As the data could not be normalized for all variables, non-parametric two-tailed Wilcoxon matched-pair signed-rank tests were used to compare overall behavioural responses between the paired exemplars. Spearman rank correlations were used to test for relationships between hind age and behavioural responses. Statistical tests were performed using Statistical Package for the Social Sciences (SPSS 18.0.0 2009; SPSS, Inc., Chicago, IL, USA) and used 0.05 levels of significance.

Results

Eleven of 15 (73.33%) sika deer hinds entered at least one proximity zone. Although 5 of 11 hinds entered each proxim-

ity zone an equal number of times, 4 of 11 hinds entered the heterospecific zone more times and 2 of 11 hinds entered the conspecific zone more times. Additionally, 5 of 11 hinds spent more total time in the non-conspecific zone and 6 of 11 hinds spent more time in the conspecific zone. Sika deer hinds exhibited very similar levels of preference response variability to conspecific and heterospecific calls (conspecific/heterospecific: IQR of instances entering zones = 3.50/3.50, IQR of total time in zones = 51.11/44.18 s; Table 2; Fig. 2). Overall, there were no significant differences in the number of instances the hinds entered the conspecific or heterospecific proximity zone ($Z_{15} = -0.63$, $P = 0.527$), or in the total amount of time spent within these proximity zones ($Z_{15} = -0.27$, $P = 0.79$) (Fig. 2a,c). Additionally, sika deer hinds did not look towards the speaker broadcasting conspecific sika deer calls for more instances ($Z_{15} = -0.74$, $P = 0.461$) or more total time ($Z_{15} = -0.51$, $P = 0.609$) than towards the speaker broadcasting heterospecific red deer calls (Fig. 2b,d). Lastly, no significant correlations were found between hind age and behavioural responses (Table 3).

Discussion

We predicted that oestrous sika deer hinds would show little flexibility in preference responses to paired presentations of conspecific versus heterospecific male mating calls with females overall preferring conspecific calls. However, females showed high inter-individual variation in behavioural responses to the stimuli with no overall preference for either call type (Table 2). These responses indicate that vocal behaviour does not form a solid barrier to hybridization for female sika deer as it does not act as a reliably strong deterrent to preference behaviours. In contrast, there was much less flexibility in responses by oestrous red deer hinds to similar acoustic stimuli, with an overall preference for conspecific calls over heterospecific sika deer calls (Wyman *et al.*, 2011). Although we acknowledge that larger sample sizes may reduce possible type II errors, comparable sample sizes were sufficient to produce significant results in similarly designed studies of hind preference behaviour (Charlton *et al.*, 2007; Reby *et al.*, 2010). Similar to our results in red deer (Wyman *et al.*, 2011), our examination of sika deer attention behaviours (number and duration of looks towards speakers) indicated that conspecific calls from unfamiliar males did not evoke more or less atten-

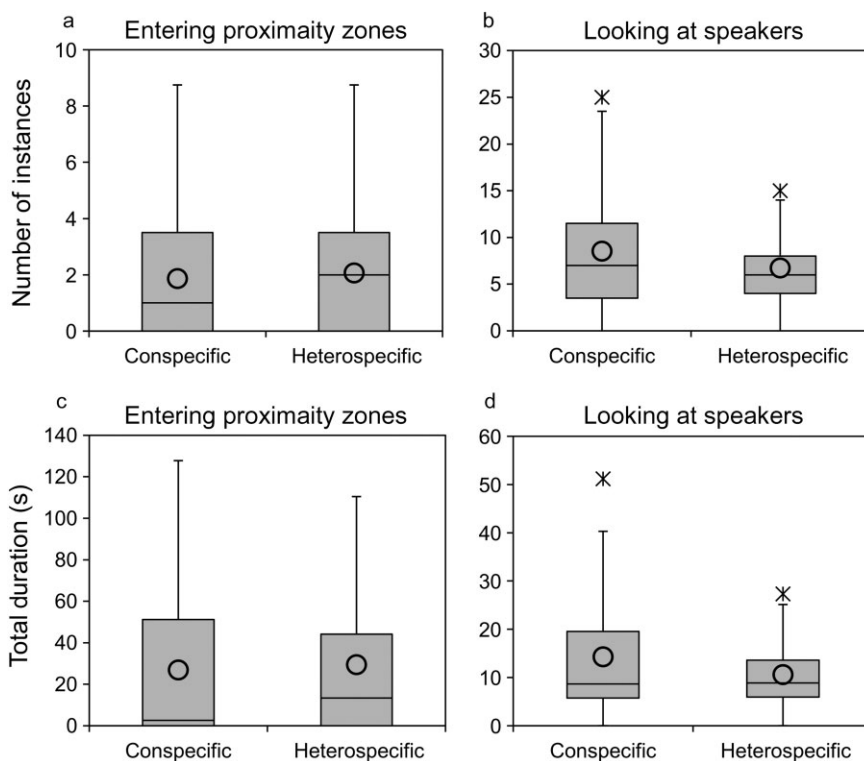


Figure 2 Behavioural responses by sika deer hinds to conspecific and heterospecific male mating calls. Box plots depict the distribution of preference and attention behaviours: (a) number of instances of entering proximity zones, (c) total time within proximity zones, (b) number of instances of looking at the speakers and (d) total time looking at the speakers. Upper and lower whisker limits are set to $1.5 \times$ interquartile range above and below the third and first quartile, respectively. Circles represent mean preference behaviour values and stars represent outliers present outside of the whisker limits.

Table 3 Spearman rank correlations between age and behavioural responses of sika deer hinds

Behavioural measure		Conspecific stimuli (r_s coefficient, P -value)	Heterospecific stimuli (r_s coefficient, P -value)
Entering proximity zones	Instances	$r_s(13) = 0.048$ $P = 0.866$	$r_s(13) = -0.140$ $P = 0.619$
	Total time (s)	$r_s(13) = 0.124$ $P = 0.660$	$r_s(13) = 0.244$ $P = 0.381$
Looking at speakers	Instances	$r_s(13) = -0.133$ $P = 0.636$	$r_s(13) = -0.229$ $P = 0.412$
	Total time (s)	$r_s(13) = -0.066$ $P = 0.817$	$r_s(13) = -0.100$ $P = 0.723$

tion than novel heterospecific male calls (Table 2). Finally, there was no relationship between age and behavioural responses (Table 3).

Although male sexual calls are considered to be important elements of sexual selection in sika deer and red deer (Miura, 1984; McComb, 1991; Minami & Kawamichi, 1992; Reby *et al.*, 2005, 2010; Charlton *et al.*, 2007), they may not present strong barriers to interspecific mating for both species. Between-species comparisons indicate potential asymmetries in the acoustic species discrimination abilities of red deer and sika deer hinds in situations of first contact between allopatric populations. Species-based asymmetries in acoustic species discrimination and interspecific mating preferences have also been documented in several other phylogenetic lineages (insects, Shapiro, 2001; Izzo & Gray, 2011; anurans, Gerhardt,

1974, 2001; and birds, Derégnaucourt & Guyomarc'h, 2003). For example, Derégnaucourt & Guyomarc'h (2003) demonstrated that female European quail *Coturnix coturnix coturnix* responded more to male mating calls from conspecifics than from reproductively compatible Japanese quail *Co. coturnix japonica* or hybrids, while in contrast, female Japanese quail responded equally to all playback stimuli.

In the context of our controlled experiments with allopatric populations, the apparent asymmetry in species discrimination abilities may be influenced by several factors, including phylogenetic history, current intraspecific selection pressures, and use of multiple cues for mate choice and species discrimination. Pre-existing neurosensory biases within closely related species may trigger attraction behaviours in response to acoustic parameters of ancestral call types (Ryan & Rand,

1993b; Hill, 1994; Endler & Basolo, 1998). In túngara frogs, it is thought that pre-existing biases cause females to not discriminate between conspecific males and their most recent ancestor, despite wide acoustic differences in male mating calls (Ryan & Rand, 1999). A phylogenetic analysis based on male vocal behaviour in Cervidae indicates that high-pitched male sexual calls probably evolved from more low-pitched ancestral male sexual calls (Cap *et al.*, 2008). Furthermore, molecular phylogenies indicate that high-pitched sika deer and wapiti *Ce. canadensis* species are more closely related to each other than to the lower pitched Western red deer species from which they split (Ludt *et al.*, 2004). Therefore, despite the evolution of higher pitched male sexual calls in sika deer, sika hinds may have retained some sensory biases for lower pitched call elements in the more ancestral-like red deer calls, which trigger initial preference responses in some female sika deer. In contrast, the European red deer hinds are not likely to have pre-existing biases for the extreme high-pitched male mating calls, which evolved later in their sister species. Interestingly, oestrous female red deer do prefer high F0 to low F0 within conspecific mating calls (Reby *et al.*, 2010), indicating that directional selection for high-pitched calls through mate choice may be emerging in this species as well.

Sika deer hinds may also have lost the ability to discriminate between male mating calls of conspecifics and heterospecific red deer over time, if the species were allopatric for long periods after speciation and thus the risk of hybridization was low (e.g. Wellenreuther *et al.*, 2009). Based on molecular analyses, the calculated time of divergence between Western red deer (from which European-based red deer subspecies evolved) and Eastern red deer (which later evolved into sika and wapiti groups) was about 7 million years ago (Ludt *et al.*, 2004), although it is unknown when these species became allopatric.

Current intraspecific selection pressures may also influence acoustic species discrimination abilities. Selection pressures may trigger female interest in the sexual vocalizations of other unfamiliar species, which contain attractive (Ryan & Keddy-Hector, 1992), exaggerated (Searcy, 1992) or novel (Elias, Hebets & Hoy, 2006) acoustic elements, although these responses are likely to change as species become sympatric. Although it is possible that some sika hinds may be attracted to elements within red deer roars due to intraspecific selection pressures, sika hinds did not consistently choose heterospecific over conspecific calls, and so specific tests of hind preference for acoustic properties within conspecific male calls need to be conducted. In red deer, strong current selection pressures on male roars may cause red deer hinds to have narrower levels of tolerance for preferred acoustic properties within calls, resulting in the rejection of sika deer moans in favour of red deer roars. Although playback experiments demonstrated that oestrous red deer hinds prefer conspecific roars with higher F0 (Reby *et al.*, 2010), the pitch of sika deer moans may be too far outside of their preference range to trigger attraction behaviours (max F0 is over eight times higher in moans than roars).

Additionally, species discrimination, using stag moans, may be weak in sika deer hinds if moans evolved to function more strongly in male competition than female choice. As a

result, sika hinds may have wide flexibility when classifying moan-like calls as 'conspecific' (i.e. red deer roars are close enough to moans to be considered conspecific), but make more detailed species or mate quality recognition decisions based on additional signals, such as other acoustic, visual or olfactory signals. The use of multiple, complex cues in mate choice decisions can evolve to reduce inappropriate matings that lower fitness (Pfennig, 1998; Candolin, 2003; Hankison & Morris, 2003). For example, in tree frogs (*Hyla versicolor* and *Hy. chrysoscelis*), females use call duration for intraspecific mate choice and call pulse rate for species discrimination (Gerhardt, 2001).

Although this study suggests that sika deer hinds may show weaker acoustic species discrimination abilities than red deer hinds to typical male sexual calls in allopatric populations, this does not mean that most initial hybridizations will occur between sika deer hinds and red deer stags. In fact, genetic evidence shows that most free-ranging hybridizations in western Scotland occur between red deer hinds and sika deer stags (Senn & Pemberton, 2009). Given the possible sensory biases, sexual selection pressures and signalling behaviours within species, the direction or likelihood of initial hybridization in free-living populations will also be influenced by a variety of local factors, such as familiarity between the populations, availability of conspecific mates, dispersal patterns, as well as biological and morphological traits (e.g. young attendance duration and body size differences) (Wirtz, 1999; Ord *et al.*, 2011). Indeed, the hybridization patterns documented in western Scotland are thought to be strongly driven by sika stags dispersing into areas populated mainly by red deer (Senn & Pemberton, 2009). The experiments described here replicate 'first contact' situations between naturally allopatric species. Ultimately, mate preferences and species discrimination abilities will probably change with increased sympatry, if fitness costs or benefits are associated with hybrid matings (Pfennig, 1998; Noor, 1999). If conspecific male traits preferred by females overlap with heterospecific traits and hybrid matings are costly, female preference may shift over time through reproductive character displacement, even at the risk of mating with lower quality conspecifics (Gerhardt, 1994; Pfennig, 2000). However, adaptive interspecific mate choice decisions may arise, if hybrid matings yield a fitness benefit (Veen *et al.*, 2001; Pfennig, 2007).

Future studies of these species should examine the behavioural responses of sympatric populations, responses to male mating calls from hybrids and the possible causes of individual differences in species discrimination abilities.

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