



Absence of Referential Alarm Calls in Long-term Allopatry from the Referent: A Case Study with Galapagos Yellow Warblers

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Abstract

Animals across diverse lineages use referential calls to warn of and respond to specific threats, and the ability to understand these calls may be dependent on experience with the threat being referenced. Yellow warblers (*Setophaga petechia*) produce referential ‘seet’ calls towards brood parasitic brown-headed cowbirds (*Molothrus ater*), which threaten the warblers’ reproductive success. Seet calls are produced frequently in populations sympatric with cowbirds, but rarely in allopatric populations, even when those populations are genetically similar, begging the question of the role of personal experience in anti-parasitic responsiveness in aggression and propensity to seet call towards brood parasites. Here we tested for seet call responses from a yellow warbler population on the Galapagos Islands (subspecies *aureola*), which has been geographically isolated from the mainland and obligate brood parasites for ~300,000 years. We presented playbacks of brown-headed cowbird calls (allopatric brood parasite), seet calls (North American yellow warbler’s referential anti-parasitic call), chip calls (yellow warbler’s general alarm call), sympatric predator calls, and harmless allopatric and sympatric control songs to breeding yellow warblers, and compared behavioral and vocal responses between treatments. We found that in response to playbacks signaling brood parasitic risk (seet and cowbird calls), Galapagos yellow warblers showed aggression comparable to controls, and much lower compared to chip or predator playbacks. Galapagos yellow warblers never produced any seet calls in response to the playbacks. Our results suggest that in geographic isolation from cowbirds, Galapagos yellow warblers do not produce or respond to referential alarm calls indicating mainland brood parasitic nest threats.

Significance Statement

Communication signals that denote specific objects in the environment, known as referential signals, are shaped by several ecologically important drivers, such as the extent of geographic overlap between signalers and referents, social learning, and direct experience with the referent. The yellow warbler is a useful focal species to explore questions about the contexts in which referential alarm calls occur because of the specificity for production of its anti-parasitic “seet” calls and because multiple populations of yellow warblers exist with varying exposure to obligate brood parasites. Our study explores referential alarm calling in a context without personal or social experience/learning (due to ~300,000 year insular separation from mainland brood parasites), and the findings are starkly different, as no anti-parasitic calls were produced at all on the Galapagos Islands, compared to mainland warblers allopatric from brood parasites for only ~6000 years, which are still able to produce referential anti-parasitic calls.

Keywords Brood parasitism · Host-parasite interactions · Playback presentations · Seet call · Nest investment · Experience-dependent behavior

Introduction

Diverse lineages of birds and mammals use referential alarm calls that convey specific information about threats to survival, such as predator type (e.g., aerial vs. terrestrial threat), size (e.g., small vs. large) and urgency (e.g., low

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vs. high risk) (Manser et al. 2002; Cäsar and Zuberbühler 2012; Townsend and Manser 2012; Gill and Bierema 2013; Suzuki 2016; Smith 2017; LaPergola et al. 2023). Referential calls are made in specific contexts that likely require prior experience and familiarity for both signalers and listeners to form a connection between the call and the referent that it denotes. For example, past research on birds and mammals suggests that usage of and responses to referential alarm calls tend to improve with experience and/or age (reviewed in Hollén and Radford 2009; Magrath et al. 2010; Gill and Bierema 2013).

Research to date suggests that exposure of signalers to the referent is crucial for the development of referential alarm calling behavior, especially in the context of geographic isolation (Avey et al. 2011; Feeney and Langmore 2013; Kuehn et al. 2016; Lawson et al. 2020). Such ontogenies are particularly well studied in avian host-brood parasite interactions, where obligate brood parasites threaten reproduction by laying their eggs in the nests of other species ('hosts') and force the parents to care for foreign offspring at the cost of their own offspring (Davies 2010). In these systems, referential alarm calls by hosts elicit anti-parasitic responses in listeners, such as enhanced aggression towards nearby brood parasites (e.g., Feeney et al. 2013) or returning to and sitting on nests (e.g., Gill et al. 1997; Gill and Sealy 2004; Lawson et al. 2021a). For example, experiments on different superb fairy-wren (*Malarus cyaneus*) populations found that populations without brood parasitic bronze-cuckoos (*Chalcites lucides*) present showed little aggression and fewer referential "whining" calls towards models of the brood parasite (Langmore et al. 2012) than populations with brood parasites. A similar study on whiteheads (*Mohoua albicilla*) found that populations in sympatry with brood parasitic long-tailed cuckoos (*Urodynamis taitensis*) produced referential alarm calls towards models of this brood parasite, but allopatric whiteheads treated nest predator and brood parasite models similarly and without referential calls (Lawson et al. 2020).

There are several mechanisms that could cause host populations allopatric with brood parasites to either reduce alarm calling behavior towards brood parasites, or cease referential alarm calling together. First, studies on superb fairy-wrens and brood parasitic bronze-cuckoos suggest that *social learning about brood parasitism* is important for the anti-parasitic behaviors to develop and populations that are not exposed to brood parasites lack such behaviors (Feeney and Langmore 2013). Or, *direct experience with brood parasitism* may be necessary for hosts to produce referential alarm calls, such that individuals from allopatric populations could be naïve and therefore unresponsive to brood parasites (discussed in Feeney et al. 2012). Last, there could be a *evolutionary divergence in referential alarm calling*,

such that allopatric populations lose the behavior altogether because of the underlying genetic differences from the sympatric populations (Feeney et al. 2012).

The interplay between social learning, personal experience, and geographic isolation on referential alarm calling behavior is particularly well-studied in the yellow warbler (*Setophaga petechia*). This North American breeding passerine is a major host of brown-headed cowbirds (*Molothrus ater*; hereafter 'cowbird'), and produces a referential 'seet' call that conspecific listeners respond to by returning to and sitting on their nests, presumably to prevent access by cowbirds (Gill et al. 1997; Gill and Sealy 2004; Lawson et al. 2021a). Studies indicate that for yellow warblers, personal experience is important for seet call development, with little evidence that social learning plays a role (Campobello and Sealy 2011). For example, Hobson and Sealy (1989a) found that older adult yellow warblers were more likely to produce seet calls and respond referentially to cowbird models compared to younger adult individuals that lacked breeding experience (Hobson and Sealy 1989a). Similarly, Campobello and Sealy (2011) demonstrated that yellow warbler females increased aggression and seet call rates towards cowbirds after removing a host egg from nests, which simulated a brood parasitism event. Key studies comparing populations of yellow warblers with or without cowbirds present further support that experience drives plasticity of seet calling behavior: populations of yellow warblers that are allopatric from the cowbird produced fewer referential calls and responded less aggressively in response to cowbird model presentation compared to sympatric populations (Briskie et al. 1992; Gill and Sealy 2004; Kuehn et al. 2016). Genetic variation is unlikely to account for differences in seet calling behavior between populations, as there is evidence of extensive gene flow between yellow warbler lineages across North America (Boulet and Gibbs (2006).

Here, we tested for behavioral evidence of seet calling with playback experiments on a yellow warbler subspecies that has not been tested before, *Setophaga petechia aureola*, which resides on the Galapagos Islands, Ecuador. This subspecies is genetically distinct from North American populations, and has lived allopatrically from cowbirds and other obligate brood parasites since establishing on these islands ~300,000 years ago (Browne et al. 2008; Chaves et al. 2012). This is a useful system to further explore how geographic isolation influences referential alarm-call development in the yellow warbler, because previous tests were on mainland populations with some potential to interact with cowbirds based on range overlap and migration patterns (Briskie et al. 1992; Gill and Sealy 2004; Kuehn et al. 2016). We conducted our playback experiment on two of the Galapagos Islands, Santa Cruz and Floreana, in order to test alarm calling responses to both brood parasitic and predatory threats. We measured behavioral responses of yellow

warblers to playbacks of allopatric brown-headed cowbird chatters, allopatric yellow warbler seet calls and two controls: sympatric small ground finch (*Geospiza fuliginosa*) songs and allopatric wood thrush (*Hylocichla mustelina*) songs. We also presented playbacks of non-parasitic threats, specifically allopatric yellow warbler chip calls (a general anti-predator call in this species), sympatric predator calls (Galapagos short-eared owl, *Asio flammeus galapagoensis*), and invasive sympatric nest predator calls (smooth-billed ani, *Crotophaga ani*).

If long-term geographic isolation from cowbirds lowers the propensity to seet call, then we did not expect Galapagos yellow warblers to seet call in response to signals of brood parasitism risk (cowbird chatter and allopatric yellow warbler seet calls), as the Galapagos populations are the most isolated of any allopatric yellow warbler populations studied to date. We also predicted that Galapagos yellow warblers would exhibit low aggression (measured as number of chip calls, latency to respond, and closest approach to stimuli) towards vocal signals for brood parasitism compared to signals of other non-parasitic nest threats.

Methods

Sites and study species

Our experiment was conducted at sites on Santa Cruz Island (two highland sites, one lowland site) and Floreana Island (two highland sites, one lowland site) in the Galápagos Archipelago, Ecuador. Both study areas have previously been part of long-term annual nesting monitoring of Darwin's finch breeding biology monitored by the Kleindorfer group (Kleindorfer et al. 2021; Common et al. 2022). The Santa Cruz highland study plots were located in the Los Gemelos area (-0.625982, -90.384829) and the lowland study plots in the El Barranco area (0.739068, -90.301467). The Floreana Island highland study plots were located in the Cerro Pajas (-1.299829, -90.455674) and Asilo de la Paz (-1.313595, -90.454935) areas, and the lowland sites near the Loberia area (-1.282974, -90.49208).

Genetic estimates place the Galápagos yellow warbler on the Galápagos Islands ~300,000 years ago, most likely originating from the mainland Central American lineage (Browne et al. 2008; Chaves et al. 2012). Galapagos yellow warblers are non-migratory and present year-round on almost every island in the Galápagos Archipelago. While there is high genetic divergence between the Galápagos lineage compared to the Central American and North American lineages, there is low divergence between islands, suggesting high gene flow between islands in the Galápagos (Browne et al. 2008; Chaves et al. 2012). Galápagos yellow warblers

breed during the wet season (December through May) with peak breeding February through April.

Determining nesting status

We conducted playback trials on active yellow warbler territories during February 2022. Sites were systematically searched every two days for the presence of male and female pairs and active nests. We verified nest stage prior to trials by checking the nest to confirm its content, and only tested the pair if their nest was in the laying or incubation stage, when yellow warblers are most likely to respond to cowbirds with seet calls (Gill and Sealy 1996; Gill et al. 2008; Lawson et al. 2021b). If a nest could not be located, we used rules from our previous studies to determine if a pair was likely to have a nest in the laying or incubation stage (Lawson et al. 2021a, b). Briefly, a male and female pair: 1) needed to be active on the territory over a three-day period, 2) exhibit signs of incubation/laying but not of other nesting stages (e.g., carrying nesting material for building or delivering food for nestlings), 3) males displayed mate-guarding, a behavior commonly used to assign laying status of the female in Parulidae warblers (e.g., yellow warblers: Hobson and Sealy 1989b; other Parulidae: Stutchbury et al. 1994; Chuang-Dobbs et al. 2001; Toms 2012), and 4) females exhibited nest defense behaviors (alarm-calling, perch-switching, wing-flicking, circle-flight), which have been used to assign Parulidae warbler nesting status in similar studies (Ficken and Ficken 1965; Marshall and Balda 1974; Hobson and Sealy 1989b; Mitra 1999). If these rules were met, the pair was tested.

Playback stimuli construction

Our experiment utilized the methodology and some playback files (e.g., cowbird chatter, seet calls, chip calls, and wood thrush song, all recorded in North America) from our previous playback experiments on yellow warblers in North America (Illinois, USA; Lawson et al. 2021a, b). Exemplars were created for seven playback treatments: (a) female brown-headed cowbird chatters (brood parasite), (b) North American yellow warbler seet calls (cowbird-specific anti-parasitic alarm call), (c) North American yellow warbler chip calls (general alarm call given toward nest threats and during conspecific interactions; Hobson and Sealy 1989b; Gill and Sealy 1996), (d) smooth-billed ani calls (introduced sympatric nest predator, hereafter 'ani'; Jara and de Vries 1995), (e) Galapagos short-eared owl calls (sympatric adult predator, hereafter 'owl'), (f) small ground finch calls (innocuous sympatric heterospecific control, hereafter 'finch') and (g) wood thrush songs (used to control for allopatric novelty of the cowbird chatter). We chose the wood thrush as a control for geographic novelty because this species effectively served as an innocuous heterospecific

control in playback experiments on North American yellow warbler populations (Kelly et al. 2019; Lawson et al. 2021a, b), The short-eared owl served as an adult predator stimulus, as it is the only native predator of songbirds on Floreana Island (Kleindorfer et al. 2021; other predators include introduced rats, cats, and anis), and one of the two native raptors on Santa Cruz Island, Galapagos. Smooth-billed anis were introduced to the Galapagos in the 1960s, colonizing nearly every island by the 1990s (see review by Cooke et al. 2019). They were used as a nest predator stimulus, as they have been observed robbing nests of eggs and nestlings of many endemic passerines, including yellow warblers (Jara and de Vries 1995; Wiedenfeld 2005; Cooke et al. 2019).

Ten-minute audio files for playbacks taken from Lawson et al. (2021a) were obtained from Xeno-Canto, sourced from the Midwestern United States (Illinois, Michigan, Minnesota, and Ohio), except for seet calls, which were sourced from Manitoba, Canada, via Gill and Sealy (2003). We created five exemplars per treatment using Adobe Audition CC 2019. Each exemplar was comprised of recordings from three different individuals. Each individual's recording was only used in one exemplar. For each exemplar, individual vocalizations were placed in a random order and then repeated. Intervals of silence were placed between vocalizations, ranging from two to six seconds based on rates found in natural recordings on Xeno-Canto. The calls were normalized for amplitude within each playback, and then standardized again across all playbacks using K frequency-weighting within Adobe Audition's "loudness normalization" feature. For each trial (described below), we chose one exemplar at random and broadcast at ~90 dB SPL (measured 0.5 m from speaker with a Leaton L815 digital sound level meter, using C frequency-weighting; Lawson et al. 2021a, b).

Playback experiment

We broadcast playbacks from a FOXPRO NX4 speaker, placed on the ground ~5 m from the nest (when the location was known), and recorded data from ~10 m away. If a nest location was unknown, the speaker was placed 5–6 m from the yellow warbler male's song post most commonly used based on our daily territory observations. Playback trials and data collection occurred for 10 min, between 5:00 and 12:00 h local time. We only tested nests and territories that were ≥ 60 m apart to maintain sampling independence. Nests at this distance likely belonged to different breeding birds based on average territory size (30 m^2) of the North American lineage of yellow warblers (Kendeigh 1941; DellaSala 1986). We also waited at least 30 min between playbacks at neighboring territories to avoid biasing responses of neighbors during their trials. Each yellow warbler territory was tested three times total, with a different, randomly assigned treatment to minimize order effects. We also waited 30 min

between trials on the same territory to maintain trial independence and avoid potential habituation.

During the playback trials, we recorded the following behavioral responses from both the male and female warbler within 30 m of the speaker (following Lawson et al. 2021a, b): (a) response latency (within 5 s increments after the start of trial when the individual was within 5 m of the speaker, or began displaying aggressive behavior/alarm calls within 30 m of the speaker: posturing, hopping, alarm calling, or attacking the speaker), (b) total number of seet calls, (c) total number of chip calls, and (d) closest approach to the speaker (m). Sixteen pairs could not be tested all three times due to logistical constraints. It was not possible to record data blind because our study involved focal animals in the field.

Statistical analyses

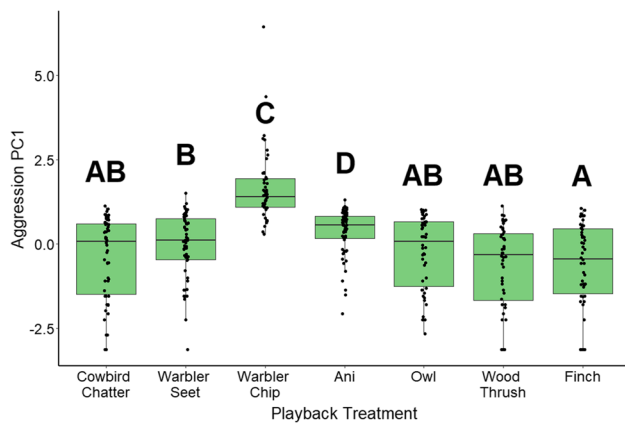
To evaluate the birds' behavior in response to playbacks, we used a principal component analysis which combined the response metrics for latency, number of chip calls, and closest approach. Seet calls were not included in the analysis because no yellow warbler ever seet called in response to any playbacks. The analysis yielded two principal components that together explained 86% of the variance in the data. PC1 explained 59% of the variance in the data and had a strong negative loading by latency to respond (-0.64) and closest approach (-0.63), and a positive loading with the number of chip calls produced (0.43). Taken together, individuals with higher PC1 scores showed stronger aggression towards the playbacks, as they responded more quickly, moved closer to the speaker, and produced more chip calls. PC2 explained 28% of variance in the data and only had a strong positive loading with chip calling (0.90), such that individuals with higher PC2 values displayed more alarm calling behavior. We used PC1 and PC2 in two separate linear models as the response variable. For PC2 we added a constant (1) to make all values positive, and then square-root transformed the data to fit a normal distribution. All models included playback treatment, sex of the subject, island (Santa Cruz or Floreana), nest status (confirmed laying/incubating nest stage or pair with unknown nest location), trial order (to account for repeated playbacks at the same territory), and playback exemplar (to account for differently sourced recordings) as fixed effects. All statistical tests were conducted in R 4.2.2 (packages lme4, emmeans, and car), with $\alpha = 0.05$. For all models yielding significant results, we conducted post hoc tests with a Tukey correction to compare the least-square means outputs between treatments.

Sample sizes

We conducted 186 playback trials on 75 yellow warbler territories, 27 of which had known nests in the laying/incubating stage

Table 1 Sample sizes of male–female pairs by treatment and whether nest location was known

Treatment	# Pairs with nests in laying/incubation stage	# Pairs with unknown nest location
Cowbird chatter	13	16
Seet calls	11	17
Chip calls	9	17
Ani calls	11	18
Owl calls	10	13
Ground finch calls	10	17
Wood thrush song	7	17

**Fig. 1** Boxplot of PC1 scores of yellow warblers in response to each 10-min playback treatment. Higher scores represent more aggressive responses by yellow warblers to the playback (lower latencies, closer approach, more alarm calls). Boxes with different letters denote post hoc statistical differences between treatments. Boxplots denote 10th, 25th, 50th, 75th, and 90th percentiles. Data points are scattered as black dots

and 48 with pairs but unknown nest locations. Table 1 provides a summary of sample sizes by treatment and territory “status” (nest location and stage known vs. nest location unknown).

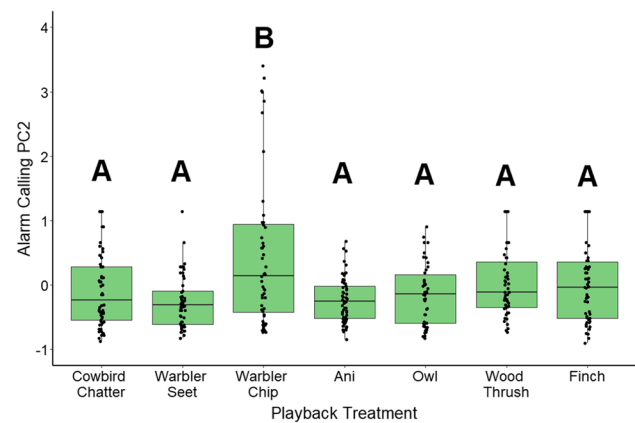
Results

Seet calling

Galapagos yellow warblers never produced any seet calls in response to any playback stimuli.

Aggression

Across treatments, yellow warblers varied considerably in their aggression (PC1) towards the playbacks (Fig. 1). Playback

**Fig. 2** Boxplot of PC2 scores of yellow warbler responses to each 10-min playback treatment. Higher scores represent more chip alarm calls made by yellow warblers during the playback. Boxes with different letters denote post hoc statistical differences between treatments. Boxplots denote 10th, 25th, 50th, 75th, and 90th percentiles. Data points are scattered as black dots

treatment (treatment term: $F_{6,185} = 29.76$, $p < 0.001$) significantly affected PC1 values, in that warblers displayed more aggression towards chip calls compared to all other treatments: cowbird chatters ($t = 10.02$, $p < 0.001$), seet calls ($t = 7.93$, $p < 0.001$), ani calls ($t = 6.41$, $p < 0.001$), owl calls ($t = 9.23$, $p < 0.001$), finch calls ($t = 11.12$, $p < 0.001$), and wood thrush songs ($t = 10.52$, $p < 0.001$). Warblers also were more aggressive towards ani calls compared to cowbird chatters ($t = 3.82$, $p < 0.01$), owl calls ($t = 3.27$, $p < 0.05$), finch calls ($t = 5.02$, $p < 0.001$), and wood thrush songs ($t = 4.61$, $p < 0.001$), but not to seet calls ($t = 1.701$, $p = 0.61$). Interestingly, warblers were statistically more aggressive towards seet calls than to the ground finch calls ($t = 3.17$, $p < 0.05$), but not to other calls.

Sex influenced aggression (sex term: $F_{1,185} = 4.58$, $p < 0.05$), with females responding more aggressively to playbacks overall compared to males. Warblers with confirmed nesting status were more aggressive to playbacks than those with unknown nest locations (status term: $F_{1,185} = 2.75$, $p < 0.01$). Additionally, warblers on Floreana responded more aggressively towards playbacks than warblers on Santa Cruz (island term: $F_{1,185} = 2.39$, $p < 0.05$).

PC1 scores were not significantly influenced by trial order (trial term: $F_{1,185} = 1.22$, $p = 0.27$) or exemplar (exemplar term: $F_{1,185} = 2.72$, $p = 0.10$).

Alarm calling

Playback treatment significantly influenced how many chip calls birds produced (PC2) during trials (treatment term: $F_{6,185} = 6.52$, $p < 0.001$) (Fig. 2). Yellow warblers produced more chips during chip call playbacks compared to all other treatments: cowbird chatters ($t = 4.27$, $p < 0.001$), seet calls

($t = 5.30$, $p < 0.001$), ani calls ($t = 5.29$, $p < 0.001$), owl calls ($t = 4.31$, $p < 0.001$), finch calls ($t = 3.57$, $p < 0.01$), and wood thrush songs ($t = 3.10$, $p < 0.05$). There were no significant differences between any other treatments.

PC2 scores were not significantly affected by sex (sex term: $F_{1,185} = 1.56$, $p = 0.21$), nesting status (status term: $F_{1,185} = 0.002$, $p = 0.96$), island (island term: $F_{1,185} = 1.57$, $p = 0.21$), trial order (trial term: $F_{1,185} = 1.65$, $p = 0.19$), or exemplar (exemplar term: $F_{1,185} = 0.03$, $p = 0.85$).

Discussion

Referential calls signal specific objects in the environment and are widely used in animal communication by birds and mammals. The use of these calls is shaped by several ecologically important drivers, such as the extent of geographic overlap between signalers and referents, social learning, and direct experience with the referent. A suite of studies on referential communication suggest that experience with the referent is critical to developing the referential alarm calling behavior and using it in the appropriate context (that is, a tighter connection between signal production and the threat that typically elicits the signal; reviewed in Hollén and Radford 2009; Magrath et al. 2010; Gill and Bierema 2013). Past studies found that experience with the referent improves appropriate usage of the referential signal, and that animals get better at using the referential signal in the correct context with age and/or experience (Gouzoules and Gouzoules 1989; Seyfarth and Cheney 1997, 2010; Hollén et al. 2008).

In the context of referential signals for brood parasites, both the fairy-wren and the yellow warbler show the same patterns, with older, more experienced birds showing stronger usage of and response to referential whining (Langmore et al. 2012) and seet calls (Hobson and Sealy 1989a) respectively. Furthermore, both species show dampened responses to these referential signals and the referent brood parasite in populations that have much lower brood parasitism risk, or are completely allopatric from brood parasites (Briskie et al. 1992; Gill and Sealy 2004; Langmore et al. 2012; Feeney and Langmore 2013; Kuehn et al. 2016). However, studies on yellow warblers in allopatry from cowbirds thus far have found that these populations still produce seet calls in response to cowbird models, albeit at very low rates (Briskie et al. 1992; Gill and Sealy 2004; Kuehn et al. 2016).

Our results suggest that Galapagos yellow warblers do not produce or recognize referential alarm calls indicating brood parasitic cowbirds at all. Warblers responded with similar aggression and alarm calling to playbacks of seet calls and cowbird chatter calls, and responses to these brood parasitic stimuli were comparable to the novel harmless control. Interestingly, warblers did display more general aggression (PC1)

during seet call playbacks compared to ground finch, the sympatric harmless control. The yellow warblers may have potentially recognized the seet call as a conspecific call, but not necessarily as signal for danger. Critically, the Galapagos yellow warblers never once produced seet calls in response to any playbacks, whereas they recognized conspecific chip calls as evidenced by their aggressive behavior and alarm calls in response (even though these too were sourced from North America).

In response to playbacks overall, female warblers on the Galapagos Islands were more aggressive than males, similar to other studies assessing sex-specific aggressive responses to threats to the nest. Furthermore, warblers with confirmed nests were more aggressive than breeding pairs that nests could not be located for. These paired birds did not show signs of nest building or chick rearing (e.g. building material or food in bill) but did display breeding behaviors (copulation, mate guarding, territory defense), such that these pairs may have had a recently failed nest, or had just paired up. This aligns with our previous study in North America, which found that yellow warblers in breeding contexts but without active nests still respond to chip calls (and seet calls in sympatry), but to a lesser extent than pairs with active nests (Lawson et al. 2021b). Galapagos warbler pairs responded most aggressively towards playbacks of conspecific chip alarm calls and smooth-billed ani nest-predatory calls compared to all other playbacks, another similarity to the North American lineage of yellow warblers, which also respond aggressively towards playbacks of nest predators and conspecific chip calls (Gill and Sealy 2003, 2004; Lawson et al. 2021a, b). Anis are known nest predators of Galapagos yellow warbler nests, but their population level impact on the warbler nest survival is unknown (Jara and de Vries 1995; Wiedenfeld 2005; Cooke et al. 2019). Importantly, the smooth-billed ani is a relatively recently introduced species and therefore a novel risk to yellow warbler nests (Cooke et al. 2020), and our findings suggest yellow warblers already do perceive the ani as a nest threat.

Perhaps surprisingly, Galapagos yellow warblers did not respond strongly to playbacks of the local Galapagos short-eared owl, a local predator of adult passerines (Grant et al. 1975; de Groot 1983), and instead responded with aggression levels comparable to the control playbacks. Studies from the perceived predation risk literature show that passerines typically reduce activity levels (including nest defense) or may flee the area altogether when presented with a predator model (Kleindorfer et al. 2005; Welbergen and Davies 2008; Feeney and Langmore 2013; Li et al. 2016). Thus, these results are consistent with predictions from parental investment theory in a life history context, which predicts that parent birds should reduce risk to themselves and maximize their own survival if they have the chance to renege and when the current investment loss is smaller than

the potential future gain (Martin 1995; Martin and Briskie 2009). Future research could explore risk perception among Galapagos songbird species towards Galapagos short-eared owls to test behavioral response to threat under different life history conditions, for example by older versus younger males (Kleindorfer 2007).

Yellow warbler populations in the Galapagos Islands have been geographically isolated from cowbirds longer than any North American populations in allopatry with cowbirds (300,000 years vs. ~6,000 years; Boulet and Gibbs 2006; Browne et al. 2008; Chaves et al. 2012). Galapagos yellow warblers are also genetically divergent from Central American and North American populations (Chaves et al. 2012). This, taken together with our results, suggest two potential evolutionary paths that led to the lack of seet calling in Galapagos yellow warblers: either 1) yellow warblers evolved the seet call prior to colonization on the Galapagos, whereafter the Galapagos lineage lost the ability to use or recognize the referential alarm call due to extended time without exposure to brood parasites, or 2) the seet call evolved in North American warblers after the Galapagos lineage had already split from the Central American lineage, such that Galapagos yellow warblers never possessed the ability to use seet calls in the first place.

It is currently unknown whether the seet call evolved only in the North American lineage, or is shared by other yellow warbler lineages. To fill this gap, it is crucial that future studies test how other subspecies of yellow warbler, such as those in Central America and the Caribbean, respond to stimuli signaling brood parasitic *Molothrus* cowbirds. These studies would also help determine whether the seet call refers to brown-headed cowbirds specifically, or any brood parasitic cowbird, as most of these other lineages are allopatric from brown-headed cowbirds, but are sympatric with bronzed cowbirds (*M. aeneus*), and/or shiny cowbirds (*M. bonariensis*), both of which are obligate brood parasites (Billerman et al. 2022). Hand-raising and common garden experiments are another important avenue for testing hosts of brood parasites in general, as these would allow researchers to account for any genetic differences between populations and test only for the role of individual and/or social experience with brood parasites. Critically, if the seet call evolved specifically to signal brown-headed cowbirds, which are only present in North America, we would expect the North American lineage to be the sole producer of seet calls. Overall, our study fills a critical gap in our knowledge of the vocal and behavioral repertoires and responses of insular yellow warblers and warrant further investigations with parallel methodologies in other, untested populations of this unique host of brood parasitic brown-headed cowbirds.

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Author's contributions SLL, MEH, and SAG designed the research; SLL wrote the first draft of the paper; SLL, FF-D and JKE collected the data with assistance from SN; SLL analysed the data; all authors edited the manuscript. All authors read and approved the final manuscript.

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Data availability The data for this manuscript are available on Figshare (<https://figshare.com/s/0a8b331b3c165c9668a6>).

Declarations

Ethics approval This study adheres to animal ethics standards in accordance with the Austrian Animal Experiments Act (§ 2. Federal Law Gazette No. 501/1989). Research for this study was also authorized by permits from the IACUC committee of the University of Illinois at Urbana-Champaign.

Competing Interests The authors declare no competing interests.

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