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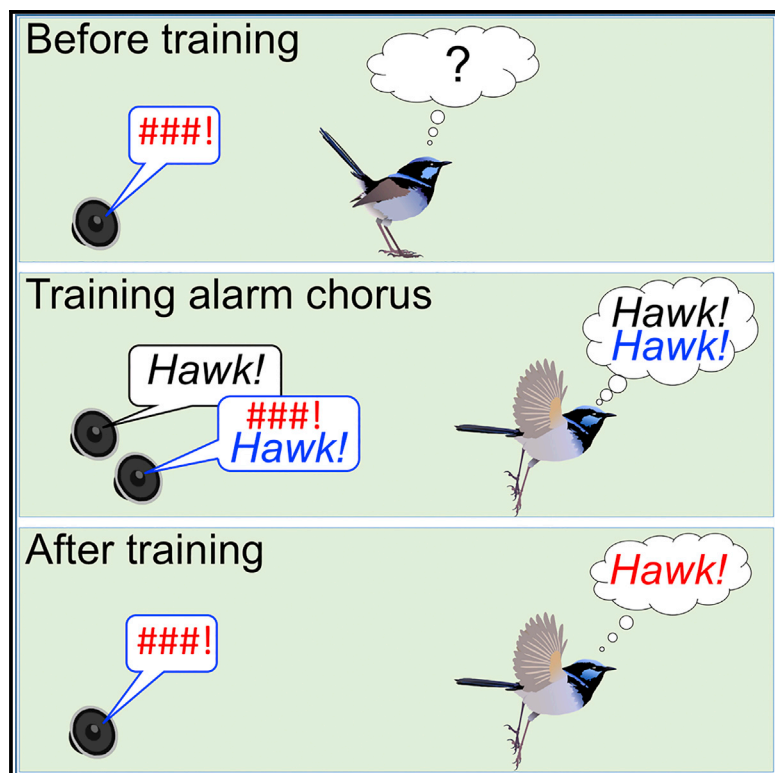
Birds Learn Socially to Recognize Heterospecific Alarm Calls by Acoustic Association

Graphical Abstract

Highlights

- Vertebrates get valuable information by eavesdropping on other species' alarm calls

- We tested if wild birds learn from other individuals to recognize new alarm calls

- Superb fairy-wrens learned to recognize new alarms by association with known alarms

- Acoustic-acoustic association meant rapid learning without even seeing a predator

Authors

Dominique A. Potvin,
Chaminda P. Ratnayake,
Andrew N. Radford, Robert D. Magrath

Correspondence

robert.magrath@anu.edu.au

In Brief

Animals often learn to eavesdrop on alarm calls of other species, but the mechanisms are unclear. Potvin et al. show that a wild bird species learns socially to recognize new calls by associating unfamiliar sounds with known alarm calls without needing to see a predator. Such acoustic-acoustic association may lead to rapid spread of recognition.

CellPress

Birds Learn Socially to Recognize Heterospecific Alarm Calls by Acoustic Association

Dominique A. Potvin,[1,3] Chaminda P. Ratnayake,[1] Andrew N. Radford,[2] and Robert D. Magrath[1,4,*]
[1]Division of Ecology & Evolution, Research School of Biology, Australian National University, Canberra ACT 2601, Australia
[2]School of Biological Sciences, University of Bristol, Bristol BS8 1TQ, UK
[3]Present address: School of Science and Engineering, University of the Sunshine Coast, Locked Bag 4, Maroochydore DC QLD 4558, Australia
[4]Lead Contact
*Correspondence: robert.magrath@anu.edu.au
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SUMMARY

Animals in natural communities gain information from members of other species facing similar ecological challenges [1–5], including many vertebrates that recognize the alarm calls of heterospecifics vulnerable to the same predators [6]. Learning is critical in explaining this widespread recognition [7–13], but there has been no test of the role of social learning in alarm-call recognition, despite the fact that it is predicted to be important in this context [14, 15]. We show experimentally that wild superb fairy-wrens, *Malurus cyaneus*, learn socially to recognize new alarm calls and can do so through the previously undemonstrated mechanism of acoustic-acoustic association of unfamiliar with known alarm calls. Birds were trained in the absence of any predator by broadcasting unfamiliar sounds, to which they did not originally flee, in combination with a chorus of conspecific and heterospecific aerial alarm calls (typically given to hawks in flight). The fairy-wrens responded to the new sounds after training, usually by fleeing to cover, and responded equally as strongly in repeated tests over a week. Control playbacks showed that the response was not due simply to greater wariness. Fairy-wrens therefore learnt to associate new calls with known alarm calls, without having to see the callers or a predator. This acoustic-acoustic association mechanism of social learning could result in the rapid spread of alarm-call recognition in natural communities, even when callers or predators are difficult to observe. Moreover, this mechanism offers potential for use in conservation by enhancing training of captive-bred individuals before release into the wild.

RESULTS

Widespread eavesdropping on alarm calls of other species relies on learned recognition, but the mechanisms of learning are mostly unknown. In some cases, individuals respond because of similarities with conspecific calls [16–20], but alarm calls vary greatly among species and are often not recognized unless familiar [9, 11, 12, 21–23], which implies learning (review [6]). The only experimental tests of learned recognition show that animals treat novel sounds as alarm calls after they are presented repeatedly with predator models [10, 13]. This suggests simple association of the novel sound with a predator, a form of asocial learning based on direct experience with the predator [24]. There has, however, been no test of social learning to recognize alarm calls, despite theory predicting it will be favored in this context [25]. There are many specific mechanisms of social learning, but broadly, it refers to learning that is facilitated by other individuals or the products of their presence or behavior [25, 26].

Here, we test whether individuals can learn to associate unfamiliar alarm calls with known alarm calls, a mechanism of social learning that does not require seeing the caller or detecting the predator itself. This potentially safe and effective mechanism of social learning has been repeatedly suggested [11, 15, 27] but never tested. It could be broadly important, as predators often appear only fleetingly or are cryptic but can provoke choruses of calls from different species. For example, raptors in flight provoke multi-species "aerial" alarm choruses [28], and multiple species can assemble around and give "mobbing" alarm calls to hidden terrestrial or perched predators [29]; in both cases, there are opportunities for social learning of new calls through acoustic-acoustic association. This mechanism is plausible, given that individuals can learn to respond fearfully to the sight of physical models by associating them with playbacks of conspecific or familiar heterospecific alarm calls, even when the callers cannot be seen [30, 31]. Specifically, we tested whether wild superb fairy-wrens can learn to recognize unfamiliar sounds as alarm calls after repeated association with a chorus of known aerial alarm calls, simulating calling in natural communities [28]. Individuals of this species learn to recognize aerial alarm calls of locally common heterospecifics [9, 11, 32, 33], and a previous experiment showed that they could learn to recognize unfamiliar sounds when associated with gliding model predatory birds [10].

The current experiment entailed repeated playbacks of initially unfamiliar sounds to 16 individually marked fairy-wrens at three stages: pre-training, training, and post-training (details in STAR Methods). Pre-training playbacks measured the initial response to two unfamiliar sounds to which fairy-wrens do not normally flee ("TB" [aerial alarm call of allopatric chestnut-rumped
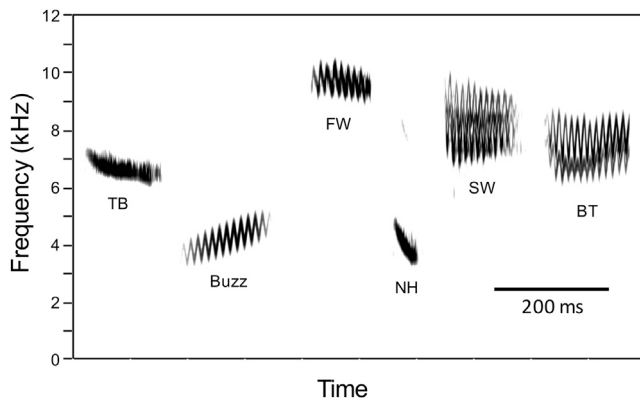
Figure 1. Sounds Used in the Playback Experiment

Spectrograms of single notes of all sound and call types used in the experiment. Unfamiliar sounds: TB (aerial alarm call of allopatric chestnut-rumped thornbill, *Acanthiza uropygialis*), Buzz (sound synthesized on computer). Known aerial alarm calls of locally common species: FW (superb fairy-wren, *Malurus cyaneus*), NH (New Holland honeyeater, *Phylidonyris novaehollandiae*), SW (white-browed scrubwren, *Sericornis frontalis*), BT (brown thornbill, *A. pusilla*). All playbacks were unique and used exemplars from different individuals. Spectrograms were produced in Raven 1.5 Pro with a Blackman window function and size of 256 samples and grid overlap 90.2% from recordings digitized at 44.1 kHz and 16 bits.

See also Audio S1.

thornbill, *Acanthiza uropygialis*] and "Buzz" [sound synthesized on computer]). One of these was subsequently used as a training sound and the other as a control sound (Figure 1 and Audio S1); the role of the unfamiliar sound was alternated between birds so that eight birds were trained to each sound. Different birds in different social groups learned to recognize these sounds in a study of asocial learning on this population in 2013 [10], so any null result would mean that social learning did not occur through the mechanism we tested, not that the sounds could not be associated with danger. That previous study also revealed no increased response to either sound after repeated playbacks to 20 individuals in the absence of predators [10], so we did not include a "sensitization" control (playbacks without known alarm calls). Training in the current experiment entailed 10–12 playbacks over 2–3 days, during which one of the two unfamiliar sounds was broadcast along with a chorus of conspecific and locally common heterospecific alarm calls (Figures 1 and 2). Training playbacks mimicked a situation in which an airborne predator prompts a chorus of aerial alarm calls, including by an unfamiliar species.

We designed playbacks to maximize realism and reduce the risk of habituation from repeated alarm-call playbacks [34]. Playbacks were in stereo with speakers separated by 3–6 m, mimicking a group of individuals, and every training playback was unique, with a variable composition of species and individuals. These features were aimed at reducing the risk of habituation. In addition, we presented three "reliability reinforcements" of the known alarm calls with a gliding model predator (pied currawong [*Strepera graculina*] or collared sparrowhawk [*Accipiter cirrhocephalus*]), which never included the unfamiliar sound (Figure 2). These reliability reinforcements were designed to ensure that the playbacks were not treated as false alarms. Focal birds fled to cover to 95.5% of the 178 chorus playbacks; 12 of the 16 birds fled to every playback, with the remaining four fleeing to between 67% and 91%. They also fled to all but one of the reliability reinforcement presentations (98%). These results show that our playback methods were successful and that the chorus of alarm calls consistently prompted a normal anti-predator response.

We assessed learned recognition during test playbacks of the unfamiliar sounds on three occasions after training: (1) day 1, 30 min to 24 hr after the last training playback; (2) day 2, the following day; and (3) week, about 7 days after training finished. The immediate response to each pre- and post-training playback was scored as a ranked variable: 0, no response; 1, glance (look for <1 s); 2, scan (look for ≥ 1 s); 3, glance or scan and then flee to cover; and 4, flee immediately to cover. We noted the presence of any conspecifics within 10 m and whether they fled to cover. Consistent with our previous study of asocial learning [10], and to account for any consolidation of learning after sleep [35], we tested for learning by scoring the individual's response on both day 1 and day 2 compared to that before training. The key prediction of social learning is that individuals would respond after training with stronger anti-predator behavior to the training sound than to the control sound compared to their response before training. We also separately assessed the stability of learned recognition over the three post-training playbacks.

Fairy-wrens learned to respond fearfully to the trained sound as if it was an aerial alarm call (Figures 3A–3C and S1 and Table S1). Birds responded more strongly to the training sound compared to the control sound after training than before training (cumulative link mixed model [CLMM] interaction between stage and whether the playback was the training or control sound: day 1, chi-squared = 5.73, df = 1, p = 0.017; day 2, chi-squared = 11.76, df = 1, p = 0.0006). Before training, birds either did not respond or glanced or scanned, whereas after training, birds fled to 81% of trained sound playbacks on day 1 and 78% on day 2 (mean 79%). By contrast, birds fled to only 38% of control sounds after training on day 1 and 19% on day 2 (mean 28%). The stronger response to the trained sound compared to the control sound shows that fairy-wrens learned socially to recognize specific sounds as alarm calls and did not simply increase their wariness of sounds in general.

Fairy-wrens showed no decline in their learned response over the week following training (Figures 3B–3D and S2 and Table S2). In the week following training, birds responded more strongly to the trained than control sound (CLMM, chi-square = 39.27, df = 1, p < 0.0001), and there was no interaction between the day of post-training testing and whether the playback was the training or control sound (CLMM: chi-squared = 2.03, df = 2, p = 0.36). The lack of interaction shows that the strength of response did not change in comparison to the control sound over the course of the week. Given that the unfamiliar sounds do not occur naturally at the study site, the learned response is therefore retained for an ecologically relevant period without the need for reinforcement. In a natural situation, if a new species had joined a community, then any learned response would be reinforced by further exposure.

Our experiment tested for social learning through acoustic-acoustic association of unfamiliar with known alarm calls, but wild fairy-wrens are often near other group members and might additionally have learnt from observing the fearful behavior of
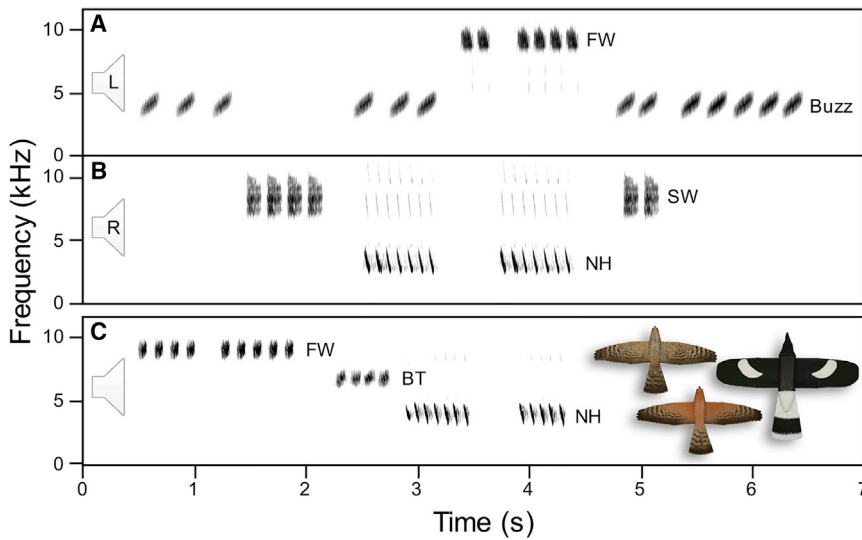
Figure 2. Examples of Playbacks Used during the Experiment

(A and B) Spectrograms of (A) left and (B) right tracks that broadcast an unfamiliar sound together with a stereo chorus of aerial alarm calls from three known species. The left track (playback set 1) includes the unfamiliar Buzz sound and FW alarm, while the right track (playback set 2) includes SW and NH alarms.

(C) Playback of aerial alarm calls of three known species—FW, BT, and NH—that was broadcast simultaneously with presentation of one of the life-sized predator models (inset, left to right: juvenile and adult collared sparrowhawks and pied currawong); there was no unfamiliar sound. These were used in reliability reinforcement playbacks. Individuals received a unique chorus on every playback during training.

Figure 1 shows call detail and gives full names of species.

others. We therefore tested whether an individual's response after training was affected by the proportion of training playbacks in which a conspecific fled to cover; individuals already in cover, or absent, were classified as not fleeing. Overall, conspecifics fled during a mean of 33% of training playbacks (SD = 24%, range 0%–73%, n = 16 birds). We detected no significant effect of conspecific behavior during training on the focal bird's response to training playbacks after training (Spearman's rank correlation: day 1, r_s = 0.16, p = 0.57; day 2, r_s = −0.10, p = 0.72; week, r_s = 0.34, p = 0.20; Figure S3). These results suggest that direct observation of the fearful behavior of others was not necessary for the learned recognition by fairy-wrens. Evidence from other studies implies that individuals are likely also to learn through associating novel sounds with the fearful behavior of conspecifics and heterospecifics, just as individuals can learn to recognize physical threats by associating them with anti-predator behavior [30, 36–40]. The lack of an effect in our study was perhaps because there was a stronger association of unfamiliar sounds with alarm choruses than fearful conspecific behavior, therefore potentially blocking or overshadowing the association with behavior [24]. Complementary studies of social learning in captivity would be useful in examining in detail the context in which different associations are formed.

DISCUSSION

Our work demonstrates that wild birds can learn socially to recognize previously unfamiliar sounds as alarm calls. Earlier work on learned recognition of heterospecific alarm calls has either focused on the occurrence of learning without addressing mechanisms [9, 11, 12, 23] or has tested for asocial learning of unfamiliar sounds through association with predator models [10, 13]. In our current experiment, learning could not be asocial because the unfamiliar sounds were never presented at the same time as predator models. Our findings are consistent with theory, because social learning is predicted to be common when asocial learning is costly, such as during learning about predators or brood parasites [5, 14, 15, 30, 36–40]. In this

dangerous context, the greater safety of social learning is likely to outweigh its disadvantage of lower reliability compared to asocial learning [25]. Social learning about predators is likely to increase fitness, with good evidence in some fish [14] and at least one species of bird [41]. An ecologically important consequence of social learning is that there can be cultural transmission among individuals and so rapid spread of behaviors through populations [25, 42].

Fairy-wrens learned to recognize sounds as alarm calls by associating them with choruses of known alarms calls. As far as we are aware, this is the first evidence of social learning through acoustic-acoustic association. Previous work has shown that alarm calls can facilitate social learning about predators themselves without being able to see the caller. For example, blackbirds (*Turdus merula*) learned to fear an unfamiliar model if they observed another blackbird mobbing it or if they heard playback of either conspecific mobbing calls or a chorus of heterospecific alarm calls [31]. Similarly, New Zealand robins (*Petroica australis*) learned to fear an unfamiliar mammalian predator model when associated with conspecific alarm calls regardless of whether a robin model was present [30], and squirrel monkey (*Saimiri sciureus*) infants learned to fear a model if associated with conspecific alarm calls [43]. Our work and these studies together suggest that familiar alarm calls can lead to associative learning about novel sounds, as well as predators.

Fairy-wrens could have learned to recognize the unfamiliar sounds through first-order or second-order conditioning. Conspecific alarm calls often prompt responses without requiring learning [44], and in some cases, this is also true of heterospecific alarm calls [6]. In these cases, the alarm calls would be unconditioned stimuli, so the learned association with an unfamiliar sound would be first-order conditioning [24, 45]. However, if there is initially first-order learned recognition of a heterospecific alarm call that itself is then associated with another unfamiliar call, this would be second-order conditioning [24, 45]. Our playback alarm choruses included both conspecific and heterospecific calls to mimic a natural situation and so retain ecological validity [46, 47], so we cannot isolate
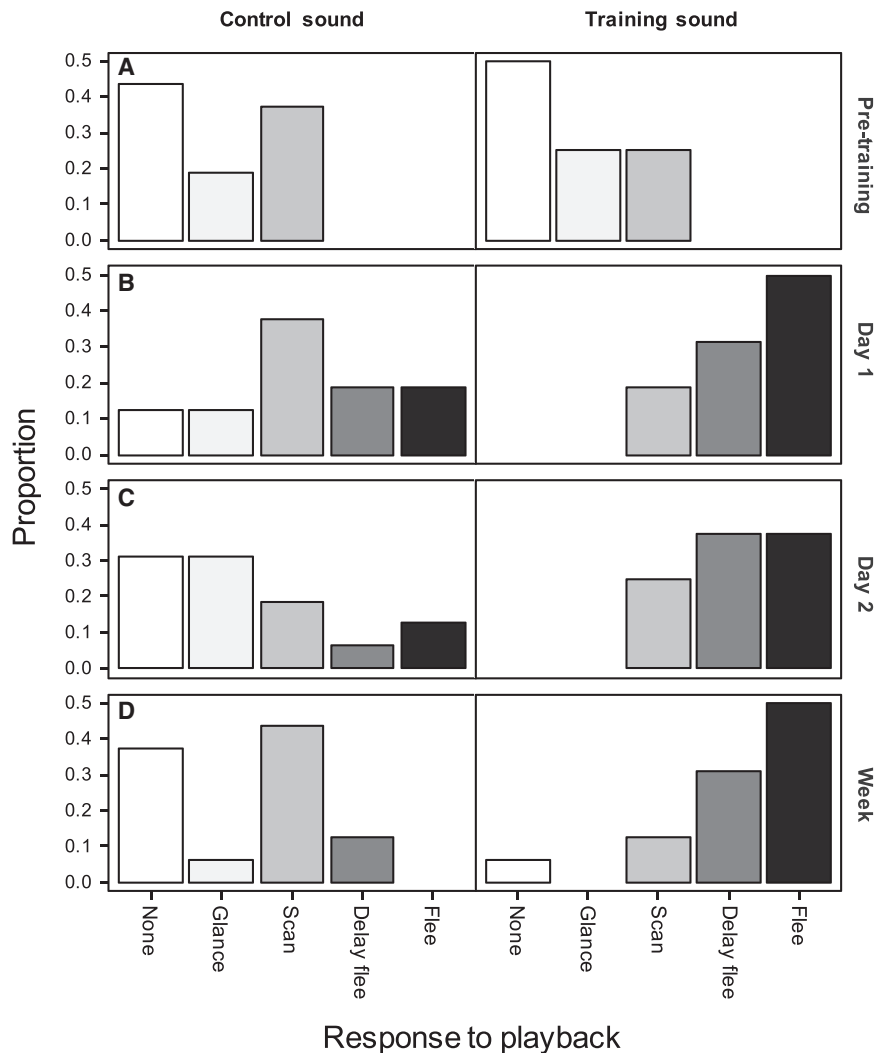
Figure 3. Response of Fairy-Wrens to Play-backs of Unfamiliar Sounds

Unfamiliar sounds were classified as control sounds if they were not broadcast to birds during the training stage and as training sounds if they were broadcast during training playbacks at the same time as choruses of known aerial alarm calls.
(A) Responses before training.
(B–D) Responses after training. (B) Day 1 (30 min to 24 hr after the last training playback). (C) Day 2 (the following day). (D) Week (about 7 days after training finished). Responses of focal birds were scored as a ranked variable: none (0, no response), glance (1, look for <1 s), scan (2, look for ≥1 s), delay flee (3, glance or scan then flee to cover), or flee (4, immediately flee to cover).
The text and Tables S1 and S2 present the results of statistical analyses, and Figures S1 and S2 show model predictions. See also Figure S3.

so simple, abrupt sounds with some similarity to conspecific alarms may be more readily recognized as alarm calls than complex, melodious ones that are dissimilar to conspecific alarms [6, 49]. Third, acoustic-acoustic association might provide a mechanism of active teaching, not just inadvertent learning. Avian vocal mimics, for example, commonly include predator calls and alarm calls of other species in combination with their own alarm calls (reviews [50, 51]), which could prompt recognition by their young of predator calls or heterospecific alarm calls [52, 53]. More broadly, mobbing alarms could prompt social learning about predators by kin [36, 37] and in multi-species choruses could facilitate recognition of heterospecific calls. Finally, acoustic-acoustic association may be valuable in conservation by providing a simple mechanism for training captive individuals before release in the wild, where many are taken by predators [15]. Training individuals to recognize alarm calls of species common at release sites should provide early warning of danger, does not require recognition of each species of predator, and could lead to learned recognition of predators themselves.

In conclusion, we found that fairy-wrens can learn socially to recognize heterospecific alarm calls through a process of acoustic-acoustic association. Trained birds had a much stronger response to the trained sound compared to the control sound, which shows learned recognition of specific sounds as alarm calls, not merely a general increase in wariness. They retained their learned response for as long as they were tested, which in a natural situation would give the opportunity for reinforcement. These findings on social learning have ecological validity [46, 47], given that experiments were done on wild animals, and help us understand the formation and function of "information webs" in natural communities, support a possible mechanism for teaching, and suggest conservation applications.

which calls prompted learning. However, fairy-wrens can learn to recognize heterospecific aerial alarm calls and respond to them about as strongly as to conspecific calls [9, 11, 32, 33], so both first- and second-order conditioning are likely.

Our findings highlight opportunities for future work. First, we must address the relative roles of asocial and social learning in heterospecific eavesdropping and whether mechanisms of social learning differ among contexts. Acoustic-acoustic association should be important when other prey species are difficult to observe and predators are difficult to detect, such as heterospecifics calling from an elevated stratum of the habitat and warning of uncommon, fast-moving, or cryptic prey [48]. Understanding learning mechanisms will help predict the maintenance and social transmission of behavior. Any one individual may rarely see a predator, so social learning may help maintain anti-predator behavior [41], and learned recognition should lead to second-order conditioning and rapid spread of both predator and call recognition. Second, it would be valuable to test if the acoustic features of unfamiliar sounds affect whether individuals can learn to recognize them as alarm calls. Learning associations can be faster if the novel stimulus is "relevant" [24],

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- CONTACT FOR REAGENT AND RESOURCE SHARING
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Study site and species
  - Ethical note
- METHOD DETAILS
  - Overview of experimental design
  - Playbacks and predator models
  - Field methods and behavioral scoring
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures, two tables, and one audio file and can be found with this article online at https://doi.org/10.1016/j.cub.2018.06.013.

AUTHOR CONTRIBUTIONS

R.D.M. and A.N.R. conceived the project, and all authors contributed to experimental design, interpretation, and writing. R.D.M. prepared the playbacks; D.A.P., C.P.R., and R.D.M. carried out the field work; and C.P.R. did the statistical analyses.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited Data		
Results of playback experiment	This paper	http://dx.doi.org/10.17632/cgthy6vvd7.1
Other		
Unfamiliar sounds and alarm calls	This paper	Audio S1

CONTACT FOR REAGENT AND RESOURCE SHARING

Further requests for reagents and resources should be directed and will be fulfilled by the Lead Contact, Robert D. Magrath (robert.magrath@anu.edu.au).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study site and species

We studied a population of superb fairy-wrens in the Australian National Botanic Gardens in Canberra ($-35.28°$ S, $149.11°$ E), where all individuals are marked with unique colored leg-bands as part of a long-term study [54]. Superb fairy-wrens are small (c. 10 g), cooperatively breeding passerines in the family Maluridae [55]. Study groups held breeding territories when we studied them, between September and December in 2015 and 2016. The Gardens include opens areas, where fairy-wrens feed on the ground, and natural and planted native vegetation, in which birds feed and seek cover from danger.

Superb fairy-wrens give distinct 'aerial' alarm calls to airborne predators and eavesdrop on the aerial alarm calls of other local passerines (Figure 1) [9, 11, 32, 33]. Common local predators include pied currawongs, *Strepera graculina*, and collared sparrowhawks, *Accipiter cirrhocephalus*, both of which prey on small birds [56, 57]. Fairy-wrens include more elements in their aerial alarm calls when danger is closer [58], and when in the open almost always flee to cover after playback of conspecific multi-element alarms [58, 59]. Fairy-wrens also flee to cover after playback of multi-element aerial alarms given by other species resident in the Gardens, including white-browed scrubwrens, *Sericornis frontalis*, New Holland honeyeaters, *Phylidonyris novaehollandiae*, and brown thornbills, *Acanthiza pusilla* [32, 33]. Fairy-wrens responding to playback of aerial alarm calls do not themselves give alarm calls (Magrath, personal observations), unlike during playback of mobbing alarm calls [60].

Aerial alarm calls vary in acoustic structure among locally common species and other members of the superfamily Meliphagoidea (Figure 1) [11, 17], and three lines of evidence show that fairy-wrens usually have to learn to recognize heterospecific aerial alarm calls. First, fairy-wrens do not respond to playback of unfamiliar alarm calls of allopatric species unless they are very similar acoustically to their own [17]. Second, fairy-wrens respond to white-browed scrubwren and noisy miner, *Manorina melanocephala*, aerial alarm calls only in locations where those species are common, where birds have the opportunity to learn, but not in allopatry [9, 11]. Third, individual fairy-wrens can be trained to respond to unfamiliar sounds as if they are aerial alarm calls by repeatedly presenting playback of those sounds with gliding model predatory birds [10].

Ethical note

All protocols were approved by the Australian National University Ethics Committee, and were designed to minimize the number of birds, and disturbance and stress. Gliding model predators were used to prompt alarm calls and as part of 'reliability reinforcement' presentations during training, so that no bird was exposed to real predators. Birds were never captured, because they were already color-banded as part of another study. Individual birds were exposed to only two pre-training test playbacks, and these same two playbacks on three occasions after training, with each lasting less than 1 s. Training playbacks entailed choruses of alarm calls, and did cause birds to flee to cover but, as in previous studies of fairy-wrens at the study site, individuals usually returned to feeding in well under 1 min [58, 61]. The number of training sessions was slightly more than in our previous study of asocial learning (10–12 versus eight) to account for potentially slower learning from social cues than direct experience. For a similar reason, we trained 16 birds compared to 10 in our previous study; in both cases, it was the minimum we judged to provide an adequate sample for statistical analyses.

METHOD DETAILS

Overview of experimental design

We tested experimentally whether wild fairy-wrens could learn socially to recognize heterospecific alarm calls, and specifically if they could learn by associating unfamiliar sounds with a chorus of known alarm calls, without a predator ever being present during playback of the unfamiliar sounds. The experiment entailed repeated playbacks to 16 individual fairy-wrens, including pre-training playback of unfamiliar sounds, followed by repeated training playbacks, and then post-training assessment of learning. Playbacks were carried out throughout the day, but at least 2 h after sunrise and 2 h before sunset, to ensure good light.

Pre-training

During pre-training, playbacks tested the initial response by individuals to two different unfamiliar sounds ('TB' and 'Buzz'; Figure 1; Audio S1), one of which was subsequently used as a 'training' sound, and the other as a 'control' sound. The sounds were different to any known alarm calls of local birds. The TB sound was a single aerial alarm call of the allopatric chestnut-rumped thornbill, *Acanthiza uropygialis*, to which naive Canberra birds do not flee [17]. It had a monotonic descending frequency, with a peak frequency of 7 kHz. The Buzz sound was synthesized in Adobe Audition 3.0 on computer, and was frequency modulated at 80 Hz and had a carrier frequency ascending from 3.4 to 4.8 kHz. It was designed to have the broad characteristics of an alarm call but without being similar to the other unfamiliar sound, or to the alarm calls of fairy-wrens or any local species [10]. One bird was excluded from the experiment because it fled to playback of the TB sound before training, but otherwise birds either did not respond or merely looked up. This low incidence of fleeing to these unfamiliar sounds is similar to our previous learning study, in which 2/32 birds (one to each sound) fled to playbacks before training [10].

Training

Individuals were trained by playing back one of the two unfamiliar sounds along with a chorus of known alarm calls on 10–12 occasions over 2–3 days, to see if birds could learn to recognize the unfamiliar sound as an aerial alarm call. Training playbacks mimicked a situation in which an aerial predator prompts a chorus of aerial alarm calls, including by an unfamiliar species. To increase realism and reduce the risk of habituation, playbacks were in stereo with speakers separated by 3–6 m, and every training chorus was unique and contained a variable composition of species and individuals (details below). Eight birds were trained to TB and had Buzz as the control, while the other eight were trained to Buzz and had TB as the control. The control sound was used to ensure any increased response after training entailed recognition of a specific sound, not simply increased wariness. The role of the sounds was swapped in case there was any underlying difference in response to the two sounds, although no difference was detected in the previous study of asocial learning [10].

One potential problem of repeated alarm-call playbacks without a predator being present is that individuals might learn that these alarm calls were unreliable, and so cease to respond [34]. If that were the case, then the unfamiliar sounds would be associated with the absence of danger and not its presence. This problem is ameliorated because the alarm-call chorus was different for each playback (above), and because we used alarms of locally common species, and so focal individuals would also be exposed to natural alarms prompted by predators. In addition, we presented three 'reliability reinforcement' presentations of the known alarm calls with a gliding model predator, after the 3rd, 6th and 9th training playbacks. These playbacks never included the unfamiliar sound, so birds could not directly associate the sound with a predator.

Post-training

Individuals were tested for their response to playback of both the training and control sounds on three occasions after training: (1) Day 1, 30 min to 24 h after the last training playback; (2) Day 2, the following day; and (3) Week, 7 days after training finished. Poor weather and other constraints meant that two Day 2 playbacks were done two days after the Day 1 playbacks, and Week playbacks ranged from 6 to 10 days after training finished (mean 7.4 days). Post-training playbacks were carried out in the same order as pre-training playbacks, to maintain perfect balance of order across birds.

Playbacks and predator models

For pre- and post-training tests, the single TB and Buzz elements were composed into 4-element calls, using Raven Pro 1.4 [62], and broadcast from a single speaker and playback set (Set 1). The total duration of these test playbacks was about 1 s. A 4-element call was used to maintain realism because local passerines use multi-element calls when danger is close, and 4-element alarm calls from familiar species almost always prompt fairy-wrens to flee to cover [32, 33]. Playbacks were calibrated so that elements were broadcast at 62 dB at 6 m, which is within the natural range of alarm calls of local species (57–70 dB [32, 33]). Calibration was carried out by re-recording playbacks along with a test tone, the amplitude of which was simultaneously measured with a Brüel & Kjær 2240 sound-level meter. Element amplitudes were measured in Raven Pro 1.4 and adjusted as required to result in the target playback amplitude.

Training playbacks were carried out in stereo, with every chorus designed to have a unique composition of aerial alarm calls, including variation in the number and type of species (Figures 2A and 2B). We made 12 unique stereo choruses. Playback Set 1 broadcast tracks that always included a sequence of the TB or Buzz elements, including a sequence of 4-elements with the same tempo as the test call. Nine of 12 also contained the aerial alarm call of one local species: superb fairy-wren, white-browed scrubwren, brown thornbill or New Holland honeyeater. In those cases, the alarm call was timed to occur immediately before, after or in the middle of a sequence of the unfamiliar sound, to reduce further any risk of habituation and to mimic natural variability. The total duration of Set 1 tracks ranged from about 4 s, if only the unfamiliar sound was broadcast, to 6 s if it also contained local alarm calls. Playback Set 2 broadcast the alarm calls of two local species, except for the first training playback that contained only one.

Calls started 1–2 s after the beginning of the track and then lasted for 2–5 s depending on the specific alarm calls. Playback from both sets therefore resulted in a chorus of familiar alarm calls, together with the unfamiliar training sound. All but two of the 12 choruses contained both conspecific and heterospecific alarms, while the others included one or two fairy-wrens. Each playback set was composed of the same equipment: a Roland R-05 digital recorder, a custom amplifier, and a Peerless 810921 tweeter speaker (frequency response: 2–11 kHz), all strapped to the observer's waist.

We used gliding predator models simultaneously with playback of familiar alarm calls during the 'reliability reinforcement' presentations (Figure 2C). Models were of life-sized juvenile and adult collared sparrowhawks, and a pied currawong (details in [10]). Most individuals received all three types of model, although three birds received only two different models. Playbacks were all unique and contained alarm calls from two or three local species, including one from a fairy-wren, and lasted about 6 s. Broadcasts were from a single speaker and carried out at least 30 min after the 3rd, 6th and 9th training playbacks.

Aerial alarm calls used in playbacks were recorded from local birds using Sennheiser ME66 directional microphones and Marantz 661 or 670 recorders digitising wave files at 44.1 kHz and 16 bits. Calls were recorded from within 10 m and prompted using gliding model hawks or currawongs. We used only good quality recordings, with a high signal-to-noise ratio and no prominent background sounds. To reduce further the risk of habituation, we varied both the timing and amplitude of elements within calls, within the natural range of variability of local species (above).

Field methods and behavioral scoring

Only one fairy-wren within any group was trained, and playbacks were done clear of territory boundaries and never when members of other groups were detected nearby, to minimize the chance that individuals on adjacent territories could hear playbacks. Focal birds were different to those included in the previous fieldwork on learning in 2013 [10]. We avoided both of the trained individuals from 2013 that remained in the study site, and avoided two other groups that contained an individual that had been resident as a non-focal bird on a territory during training in 2013. Furthermore, given that neither training sound occurred naturally at the study site, there was no possibility of non-experimental learning within the population.

Pre- and post-training playbacks were carried out and scored in the same way. Playbacks were conducted when the focal bird was about 10 m away (mean 10.3 ± SD 1.4 m, measured with a Bushnell 1300 ARC rangefinder; n = 128 playbacks to 16 birds), in clear view in the open, on or near the ground (0–1.5 m), and at least 0.5 m from cover (range 0.5–7 m). Each playback was preceded by at least 5 min of undisturbed behavior, with no territorial interactions, no predators, and no alarm calls by conspecifics or heterospecifics. The focal bird was either alone or the closest bird in the open. The immediate response to playback was scored as a ranked variable: 0, no response; 1, glance: look for < 1 s; 2, scan: look for ≥ 1 s; 3, glance or scan and then flee to cover; 4, flee immediately to cover. We noted the presence of any conspecifics within 10 m, and whether they fled to cover.

Training stereo playbacks entailed two people who were 3–6 m apart and about 10 m from the focal bird. The playback was initiated from Set 1 ('left' speaker in Figure 2), while the person with Set 2 ('right' speaker in Figure 2) initiated playback as soon as they heard the start of the Set 1 playback. This led in all cases to an overlap in time of playbacks from the two speakers, and so a chorus of calls that lasted for about 6 s. We scored whether the bird fled to cover during playbacks to determine whether there was a consistent response.

Reliability reinforcement entailed coordinating playback of familiar alarm calls and presentation of a predator model. To do so, one person started the playbacks, and about 2 s later a second person threw the model so that it glided toward and past the focal bird. The model was therefore airborne during the remaining 4 s of playback. Presentations were only done when the focal bird was in clear view, and out of cover, to ensure that it had the opportunity to see the model. Again, we noted whether the focal bird fled to cover.

QUANTIFICATION AND STATISTICAL ANALYSIS

Our primary test for learning was based on the ranked response to playback before and after training, so we used Cumulative Link Mixed Modeling (CLMM) [63], implemented using the clmm function in the 'ordinal' package in R 3.4.4 [64–66]. Bird identity was the random term, and playback role (trained versus control) and stage (pre- versus post-training) were the main explanatory terms, with ranked response (5 levels) as the response variable. The key prediction of learning is that individuals would, after training, respond more strongly to the trained compared to the control sound. This means that there would be a statistical interaction between playback treatment and stage. An equal increase in response to the trained and control sound would mean no interaction and would rule out learned recognition of the trained sound itself, and instead imply that birds had become wary of all sudden sounds. In addition to sound role, stage and their interaction, initial maximal models also included the sex of the focal individual, the type of training sound (TB or Buzz), and whether another bird in the group fled to cover after the playback. We included these terms as explanatory variables because sex, acoustic features of sounds, and nearby individuals can have consistent effects on responses during playback experiments, including on fairy-wrens [17, 21, 59]. None of these additional terms proved significant in tests of responses at Day 1 or Day 2 (Table S1). We used model simplification by backward elimination of least significant terms [67], tested by likelihood ratio tests, and assessed the significance of terms by dropping or adding them to the final models. Predicted responses from these final models, calculated using clmm2, are shown in Figure S1. Single-term deletions from the maximal model produced identical conclusions and very similar probability estimates for all terms. We carried out these tests for learning based on response on both Day 1 and Day 2 post-training playbacks. Our previous study of learning also used both days [10], and we included the Day 2 responses in addition to Day 1 because birds can consolidate learning during sleep [35], and all Day 2 playbacks were after at least one night.

To examine the stability of learned behavior after training we carried out similar CLMM analyses, with bird identity as the random term, comparing the ranked response to playback over the three post-training samples (excluding the pre-training playbacks). The maximal model included ranked response as the response variable, sound role (control or trained sound), stage (Day 1, Day 2 or Week), sound role by stage interaction, as well as the sex of the focal individual, the type of training sound (TB or Buzz), and whether another bird in the group fled to cover after the playback. We used the same model simplification procedure, and again none of the additional terms proved significant (Table S2). Predicted responses from the final model, calculated using clmm2, are in Figure S2. Again, single-term deletions from the maximal model produced identical conclusions and very similar probability estimates for all terms. A rapid decline in the strength of response would lead to a statistical interaction between stage (Day1, Day2, Week) and sound role (training versus control sound), whereas a lack of interaction implies no significant change over the week.

Our experiment tested whether individuals could learn to associate novel sounds with a chorus of known alarm calls, but social learning might have been secondarily enhanced by observing other members of the group flee to cover during training playbacks. We therefore tested whether responses by focal birds after training were affected by the proportion of training playbacks in which a conspecific fled to cover. This potential effect of conspecific behavior on learning during the training period is distinct from any immediate effect of conspecific behavior when testing before or after training, which was included in the CLMM models described above. To assess the effect of conspecific behavior during training on the subsequent response of trained birds, we could not use CLMM analyses because the proportion of playbacks with conspecifics fleeing was a continuous, rather than categorical, variable. Instead, we used Spearman rank correlations [68] to test for relationships between the proportion of playbacks with conspecifics fleeing and the ranked response. We used separate correlations for Day 1, Day 2 and Week responses (Figure S3).