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Appendix from T. J. Ord and J. A. Stamps, “Species Identity Cues in Animal Communication”

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Additional Details on the Robot Experiment

Supplementary Material and Methods

Robot Construction

To construct the robots (fig. A1), we made lifelike models of *Anolis gundlachi* with latex (RD407 soft-grade mask latex; The Monster Makers), using plaster of Paris casts of three adult males that were known territorial holders and were removed from the study population in 2006. Each model was painted and attached to two step motors (1.8-inch linear actuator, HIS 46000 series; Hadyen Switch and Instrument), one of which produced the up-and-down movement of the head while the other extended and retracted the dewlap. Each step motor was connected to a compact integrated driver (MicroLynx-4, MX-CS 100-401; Intelligent Motion Systems) that implemented display programs. The robot was powered by a cluster of 32 AA rechargeable batteries hidden below the robot model, and the rest of the mechanics were inside a plastic container fitted with a quick-releaser tripod plate.

Display programs were developed from data compiled from an extensive video library of *Anolis* territorial displays. We first plotted display-action-pattern (DAP) graphs (Carpenter and Grubitz 1961; e.g., fig. A2) for territorial displays of *A. gundlachi* and those of *Anolis grahami* from Jamaica. The DAP graphs were created from QuickTime clips of displays by tracing the movement of the body and dewlap frame-by-frame with ImageJ (ver. 1.34; National Institutes of Health; see Ord et al. 2007). We then inspected graphs and selected representative displays from *A. gundlachi* and *A. grahami* to use as the conspecific and novel displays, respectively. We measured the temporal and amplitude characteristics of displays from DAP graphs to an accuracy of 33 ms and 0.6–0.7 mm (this reflects the number of video frames [30/s] in the “National Television Systems Committee” [NTSC] format and the resolution of display movement [1.5–1.6 pixels/mm, determined by the distance at which focal lizards were recorded in the field] depicted in video clips used to create DAP graphs). These data were then used to write programs for each display sequence. The duration of the novel display was extended to match the length of the conspecific display (fig. A2).

We uploaded programs to robot drivers from a personal computer, using software supplied by the driver manufacturer (IMS Terminal: Integrated ASCII Terminal and Program Editor, ver. 4.1.48). We then videorecorded displaying robots and used DAP graphs and computational motion analysis (Peters et al. 2002; see Ord et al. 2007 for details) to confirm that the robots imitated the display pattern and the natural movement of live animals (fig. A2). Robots were programmed to perform displays every 88.6 s, based on the median interval between broadcast displays for 22 free-living males videorecorded from the study population in previous years.

We paid special attention to the color of the robots’ dewlaps because of the putative role the dewlap plays in species recognition in anoles (Williams and Rand 1977; Nicholson et al. 2007). We made the dewlap out of L’eggs “suntan” sheer panty hose (product 60426) lightly washed with Americana “golden straw” acrylic paint from DecoArt (DA168) that together reflected and transmitted light in a manner similar to the real dewlap (fig. A2*B*). Color spectra for the robot dewlap were kindly measured by Leo T. Fleishman, who also provided spectral data on live males of *A. gundlachi* from the same population studied here. Protocols for color measures of live animals are reported elsewhere (e.g., Fleishman et al. 1993; Leal and Fleishman 2002). Measurements of the robot dewlap were taken with a USB2000 fiber-optic spectroradiometer under a 300-W xenon arc lamp positioned 1 m away from the dewlap. The radiance probe was placed 10 cm from the surface at an angle of 30°. The room was darkened, and reflectance was measured with the probe positioned on the same side of the dewlap as the light source, whereas transmission was measured with the probe placed on the side of the dewlap away from the light source. All measurements were taken from a 2-mm-diameter spot at the center of the artificial dewlap. The reflectance of a white standard was also taken under the same protocol. Dewlap data were then converted into a relative measure to this white standard and were smoothed by taking the median value over every 2 nm. The data reported in figure A2*B* are the average amounts of light reflected and transmitted through the dewlap, the combined spectra of which is most likely how animals perceive dewlap color in the wild (L. T. Fleishman, personal communication).

As shown in figure A2, display movements and the dewlap color of robots were very similar to those of live animals and within the natural range of variation. The slight ultraviolet (UV) reflectance of the dewlap of live lizards measured in the laboratory is unlikely to be of any great (if any) functional importance because the forest canopy tends to filter out UV from the light of deep-shade environments occupied by *A. gundlachi* (Fleishman et al. 1997).

Playback Protocol

Trees for the robots were selected if they were free of conspecifics and were located at distances comparable to male-male nearest-neighbor distances in this population. The average distance to the nearest male neighbor for males at this locality was 4.8 ± 2.53 m (mean ± SD, $N = 27$ territory holders; Ord and Stamps 2008), so robot playbacks were presented at 4.2 ± 1.00 m from the focal lizards ($N = 308$ presentations). A video camcorder was used to record the behavior of the focal lizard during the experiment and was positioned several meters from the focal lizard and at least 4 m away from the robot. Once the camcorder operator began recording, an assistant switched the robot on and removed a cover concealing the model. The assistant then moved quietly away from the test area and out of view of the focal lizard.

Playback trials began with the robot remaining stationary for 7 min, allowing a 2-min period for the focal lizard to become accustomed to the experimental setup and the presence of the camcorder operator and a 5-min baseline period of observation. Once the robot began to display, it performed six displays over a 10-min period. Trials were aborted if the focal lizard moved out of sight of the robot during the opening 7-min period or was out of sight of the robot for more than 5 min during the 10-min playback period. At the end of the trial, we counted the number of adult male neighbors that were within the line of sight of the focal lizard and measured the amount of available habitat light at a lizard's perch site with a light meter, on the basis of earlier studies indicating that both of these factors affect display behavior in *A. gundlachi* (Ord and Stamps 2008). The tree of the focal lizard was also tagged with brightly colored biodegradable flags. These tags were used to ensure that playbacks were conducted at least 20 m from previous trials. A male's territory is likely to have a radius of less than 5 m (i.e., the average distance to male neighbors for this species is 4.8 m; see above; Ord and Stamps 2008), and 20 m would place the robot at least two territories away from a previous focal lizard. This distance excluded the possibility of mistakenly retesting lizards and was far enough away to ensure that successive focal lizards were out of view of previous playbacks conducted in the same study area. At no time were lizards caught, and we took particular pains to minimize the disturbance to animals throughout the study.

Analyses

Display behavior of focal lizards was scored from video with JWatcher version 1.0 event recorder and analysis software (Blumstein and Daniel 2007) by a single observer who was blind to the stimulus presented. We focused on the headbob when quantifying the responses of focal lizards because virtually all of the territorial displays of *A. gundlachi* include headbobs (with or without dewlap extension; T. J. Ord, unpublished data). We conducted three sets of analyses on the head-bobbing behavior of focal lizards to assess (i) their responses to a still robot versus their responses to the control stimulus during the baseline period, (ii) whether focal lizards responded more strongly to conspecific territorial display than to novel display, and (iii) whether response latencies differed as a function of the robot's displays. For the first two analyses, we computed two response variables: the proportion of focal lizards that performed at least one headbob display and the total time that each lizard spent head-bobbing. For analysis (i), we compared these two response variables for focal lizards presented with a still robot to those for focal lizards presented with the control stimulus. To address the second question (ii), we compared the same two response variables for focal lizards presented with different types of displays. Finally, to study response latency, we computed the time from the start of the playback period to the production of the first display by lizards and then asked whether this response variable varied as a function of the displays performed by the robotic lizards.

For measures of total time spent head-bobbing and latency to respond, we checked whether data were normally distributed, using Kolmogorov-Smirnov tests. When assumptions of normality were met, we performed an ANCOVA with factors for robot display type (conspecific vs. novel) and dewlap (extended vs. not extended) and with covariates for the distance of the robot from the focal lizard, the number of male neighbors, and the amount of available habitat light (variables that potentially affect display behavior; see “Playback Protocol” above and Ord and Stamps 2008). In instances where normality could not be met and improved with transformations of the data or for proportions data, we used nonparametric tests. For those tests examining the proportion of lizards responding, we used a χ^2 or a log-linear test with factors for the number of lizards responding, the type of display performed by the robot, and whether the robot extended the dewlap. All statistical tests were performed using SPSS 11.5 (SPSS, Chicago) for the Macintosh operating system.

We present the FDR-corrected α-level for each test alongside P values. Those P values at or below the FDR-α are interpreted as statistically significant.

Supplementary Results

Baseline Response

The proportion of lizards that displayed during the baseline period was similar in the presence of a stationary model of an adult conspecific male (still robot) and the leaf control ($\chi^2 = 1.51$, df $= 1$, $P = .22$, FDR-$\alpha =$ .017). However, a closer examination of the time spent displaying revealed that the conspecific model was in itself sufficient to elicit heightened aggressive responses from focal lizards. Specifically, the total time focal lizards spent performing headbob displays was significantly greater in the presence of the conspecific model than in the presence of a leaf control (Mann-Whitney test: $U = 1{,}911.5$, $P = .004$, FDR-$\alpha = .050$, $N_{\text{still robot}} = 94$, $N_{\text{control}} = 56$; fig. A3*A*).

Level of Response Elicited by Playbacks

Over the 10-min playback period, a larger proportion of lizards displayed in the presence of a displaying robot than in the presence of a still robot ($\chi^2 = 4.72$, df $= 1$, $P = .03$, FDR-$\alpha = .042$). Of the lizards presented with a displaying robot, the proportion of lizards that responded was larger when the robotic displays included the dewlap extension (partial $\chi^2 = 7.83$, df $= 1$, $P = .005$, FDR-$\alpha = .025$), but whether the display was conspecific or novel made no difference (partial $\chi^2 = .661$, df $= 1$, $P = .42$, FDR-$\alpha = .025$), nor was there any indication of a higher-order interaction between dewlap and display type ($\chi^2 = .661$, df $= 1$, $P = .92$, FDR-$\alpha = .025$). To examine the total time focal lizards spent head-bobbing, we controlled for the general increase in arousal prompted by the still model (fig. A3*A*) by subtracting the total time spent head-bobbing during the baseline period from the total time observed in the first 5 min of the playback period. Positive values indicated that the time spent displaying by focal lizards increased once the model started to display; zero values indicated that the display responses did not change; and negative values indicated that the display responses decreased once the model began to display. Following this adjustment, the type of display performed by the robot had no discernible effect on the amount of response of the focal lizards; lizards did not display more or less depending on whether the robot performed the conspecific or the novel display (see table 2 in the main text).

Latency of Response to Playbacks

Of the lizards that displayed during playbacks, the type of display viewed by lizards had no effect on latency to first headbob display, but robotic displays that extended the dewlap tended to be more effective at eliciting rapid first responses from focal lizards (table A1).

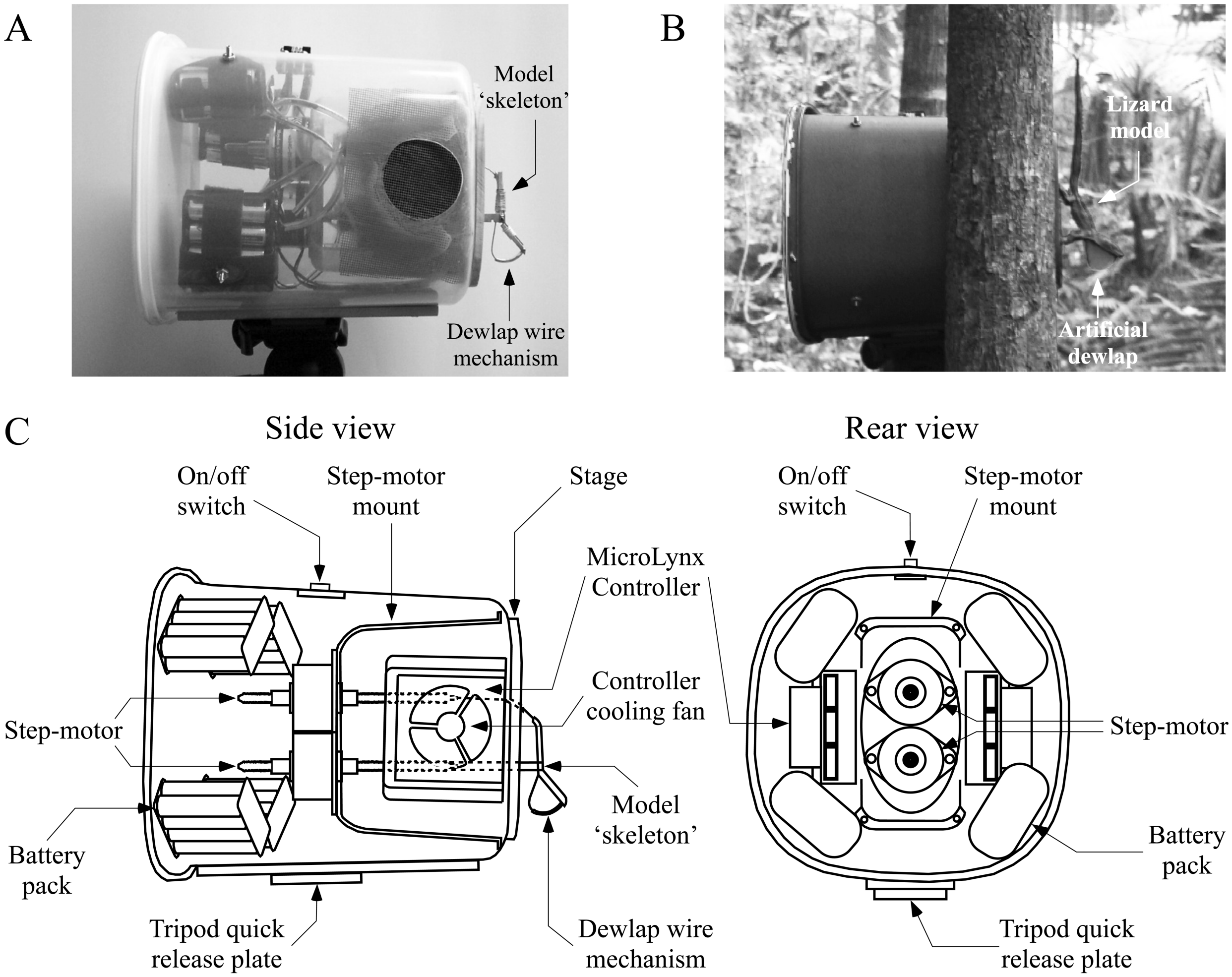


Figure A1: *Anolis gundlachi* robot. *A*, Unpainted and without the latex model and artificial dewlap. *B*, Completed robot photographed in the field middisplay and positioned on a tripod such that the model would appear to be an adult male displaying on a neighboring tree several meters away from the focal lizard. *C*, Details of the construction of the robot.

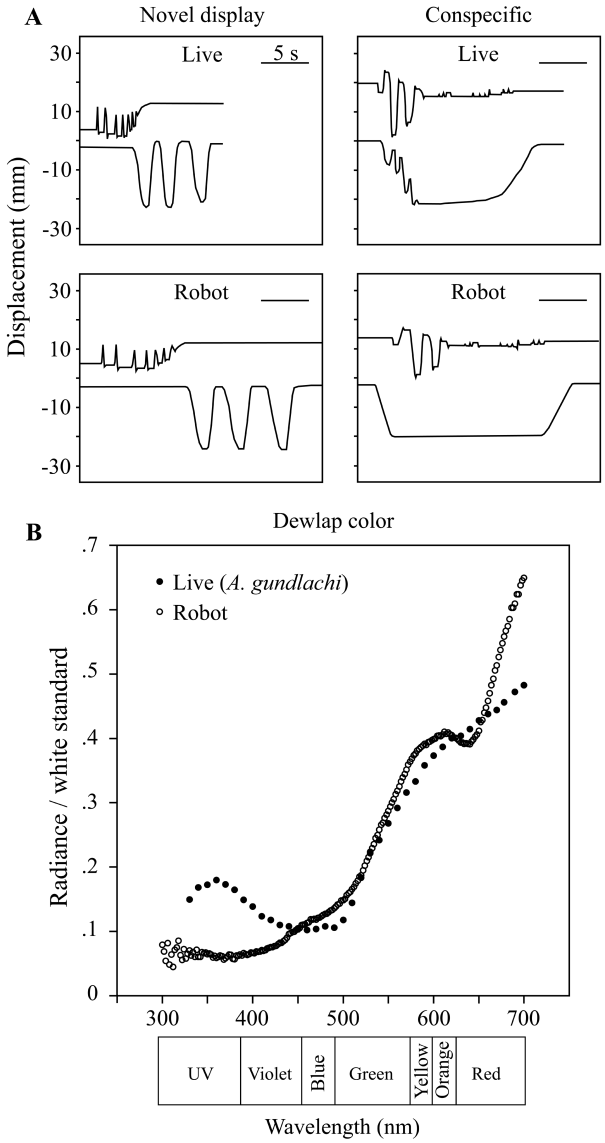


Figure A2: Comparison of the display movement (*A*) and dewlap color (*B*) of the robot to those of free-living adult male *Anolis gundlachi* and *Anolis grahami* (display movement only). Display movement is illustrated by display-action-pattern graphs showing the up-and-down movement of the body (top line in each plot) and the extension and retraction of the dewlap (*bottom line*) over time. These graphs show that the temporal and amplitude characteristics of the robot display patterns are very similar to those of free-living lizards (note: the duration of the nonconspecific novel display given by the robot was extended to match the duration of the conspecific display). Motion analysis (Peters et al. 2002) was also used to confirm that the speed of the robot display and that of actual lizards were comparable. Dewlap color shown is the average amount of light reflected and transmitted through the dewlap (see main text for details).

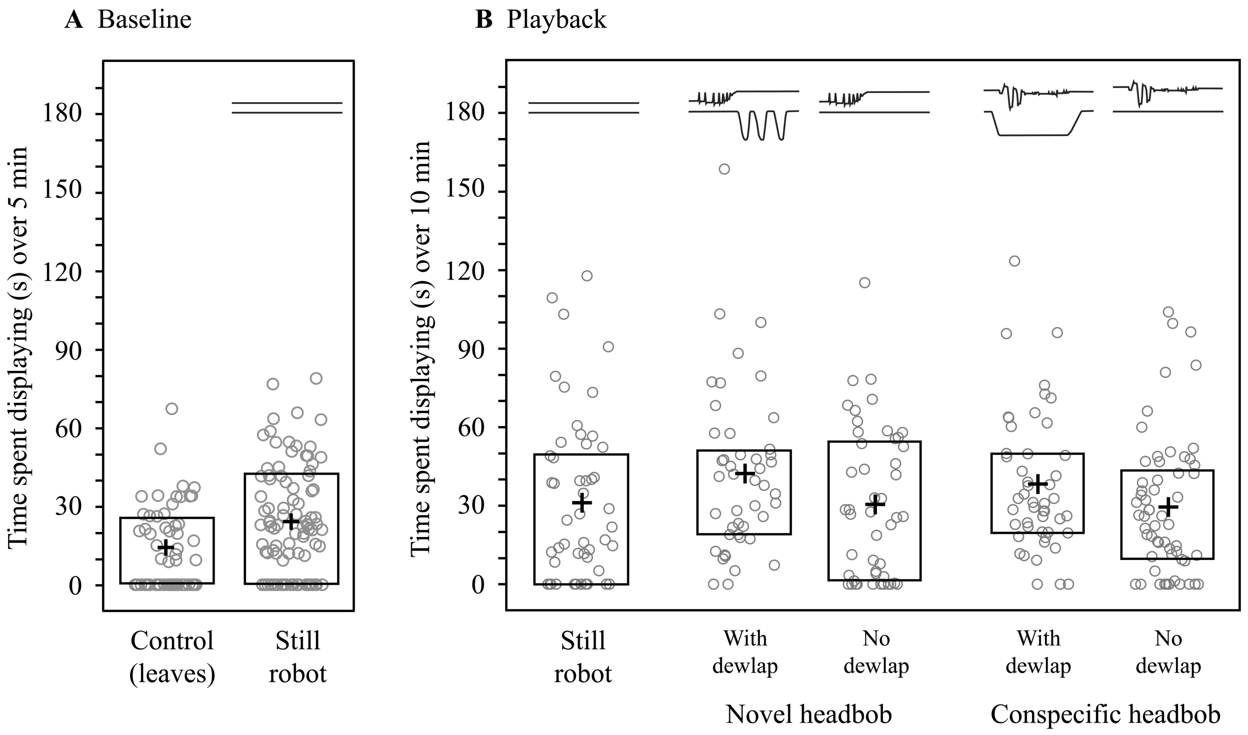
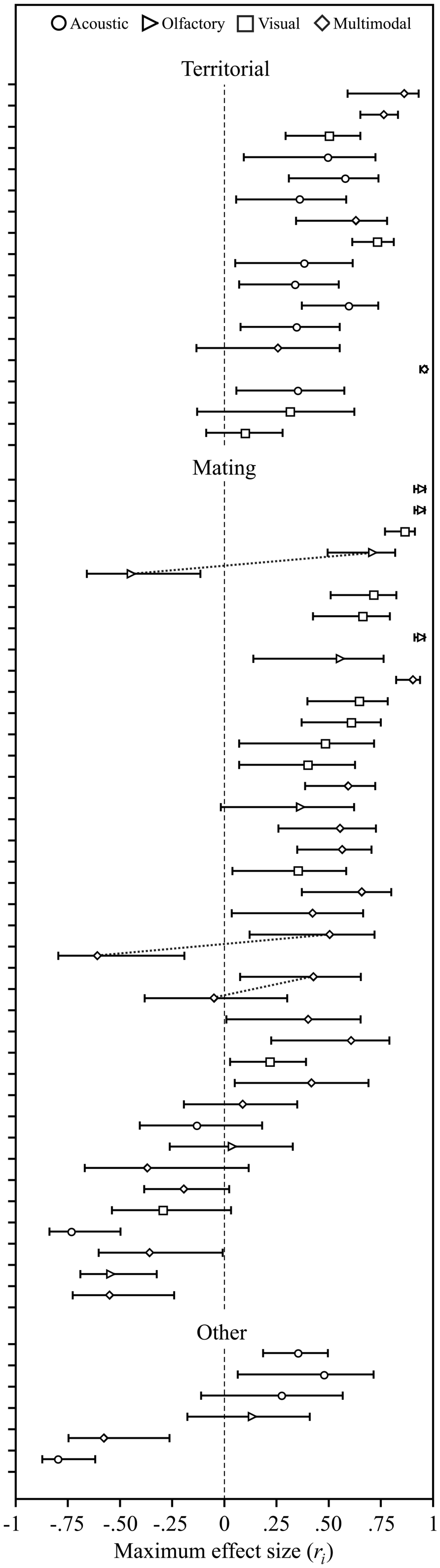


Figure A3: Total time spent by free-living *Anolis gundlachi* performing aggressive headbob displays during the initial 5-min baseline period (*A*) and the following 10-min playback period (*B*). Individual data points correspond to the total amount of display given by each focal lizard. Also shown is the interquartile range (*boxes*) and average of the responses (*plus signs*) elicited by each stimulus, along with display-action-pattern graphs showing the up-and-down movement of the body (*top line*) and the extension and retraction of the dewlap (*bottom line*) over time performed by the robot during the playback period. Analyses in the appendix indicated that more lizards responded to the displaying robot than to the still robot, confirming that focal lizards responded to the “behavior” of the robot.



Species:	Taxon:	Cue type:	Mean N:	Study type:	Source:
Territorial					
Parus caeruleus	Bird	F	18	Field	Hansen and Slagsvold (2003)
Parus major	Bird	F	21	Field	Hansen and Slagsvold (2003)
Dendroica occidentalis	Bird	F	34	Field	Pearson and Rohwer (2000)
Melospiza georgiana	Bird	F	12	Field	Nowicki et al. (2001)
Steptopelia roseogrisea	Bird	N	16	Field	de Kort and ten Cate (2001)
Serinus serinus	Bird	N	19	Captive	Cardoso et al. (2007)
Zonotrichia capensis	Bird	F	13	Field	Moore et al. (2004)
Dendroica townsendi	Bird	F	34	Field	Pearson and Rohwer (2000)
Steptopelia vinacea	Bird	N	16	Field	de Kort and ten Cate (2001)
Steptopelia capicola	Bird	F	24	Field	den Hartog et al. (2007)
Steptopelia capicola	Bird	F	20	Field	de Kort et al. (2002)
Steptopelia vinacea	Bird	F	24	Field	den Hartog et al. (2007)
Hemidactylus frenatus	Lizard	F	13	Captive	Dame and Petren (2006)
Cyprinodon variegatus	Fish	F	17	Field	Leiser (2003)
Streptopelia vinacea	Bird	F	20	Field	de Kort et al. (2002)
Icteria virens	Bird	F	10	Field	Mays and Hopper (2004)
Anolis gundlachi	**Lizard**	**N**	**48**	**Field**	**This study**
Mating					
Xiphophorus montezumae	Fish	N	13	Captive	McLennan and Ryan (2008)
Xiphophorus nigrensis	Fish	N	16	Captive	McLennan and Ryan (1999)
Pseudotropheus callainus	Fish	F	15	Captive	Couldridge and Alexander (2002)
Xiphophorus birchmanni	Fish	F	15	Captive	Wong et al. (2005)
		N	15		
Pseudotropheus zebra ('gold')	Fish	F	15	Captive	Couldridge and Alexander (2002)
Pseudotropheus lombardoi	Fish	F	15	Captive	Couldridge and Alexander (2002)
Xiphophorus montezumae	Fish	N	16	Captive	McLennan and Ryan (1999)
Laticauda frontalis	Snake	F	9	Captive	Shine et al. (2002)
Poecilia latipinna	Fish	N	18	Captive	Ptacek (1998)
Pseudotropheus zebra ('red')	Fish	F	15	Captive	Couldridge and Alexander (2002)
Xiphophorus birchmanni	Fish	N	17	Captive	Wong and Rosenthal (2006)
Icteria virens	Bird	F	10	Field	Mays and Hopper (2004)
Heterandria bimaculatat	Fish	N	16	Captive	Basolo (2002)
Poecilia velifera	Fish	N	24	Captive	Kozak et al. (2008)
Laticauda colubrina	Snake	F	13	Captive	Shine et al. (2002)
Xiphophorus pygmaeus	Fish	N	15	Captive	Hankison and Morris (2003)
Poecilia petenensis	Fish	N	24	Captive	Kozak et al. (2008)
Cyprinodon variegatus	Fish	F	18	Captive	Rosenfield and Kodric-Brown (2003)
Poecilia orri	Fish	N	12	Captive	Ptacek (1998)
Drosophila yakuba	Insect	F	21	Captive	Coyne et al. (2002)
Poecilia latipinna	Fish	F	11	Captive	Gumm and Gabor (2005)
		N	13		
Poecilia mexicana	Fish	F	13	Captive	Riesch et al. (2006)
		N	15		
Poecilia mexicana	Fish	N	18	Captive	Ptacek (1998)
Drosophila santomea	Insect	F	47	Captive	Coyne et al. (2005)
Photinus greeni	Insect	N	53	Captive	Michaelidis et al. (2006)
Drosophila yakuba	Insect	F	64	Captive	Coyne et al. (2005)
Drosophila santomea	Insect	F	21	Captive	Coyne et al. (2002)
Serinus serinus	Bird	N	27	Captive	Cardoso et al. (2007)
Plethodon shermani	Salamander	N	19	Captive	Rollmann et al. (2003)
Leptidea reali	Insect	F	11	Captive	Friberg et al. (2008)
Poecilia mexicana	Fish	N	40	Captive	Plath et al. (2008)
Cyprinodon pecosensis	Fish	F	18	Captive	Rosenfield and Kodric-Brown (2003)
Melospiza georgiana	Bird	F	10	Field	Nowicki et al. (2001)
Leptidea sinapis	Insect	F	11	Captive	Friberg et al. (2008)
Xiphophorus continens	Fish	N	24	Captive	McLennan and Ryan (2008)
Hemidactylus frenatus	Lizard	F	14	Captive	Dame and Petren (2006)
Other					
Barbary macaque	Primate	F	55	Captive	Teufel et al. (2007)
Eudyptula minor	Bird	N	10	Captive	Nakagawa et al. (2001)
Taeniopygia guttata	Bird	?	13	Captive	Braaten and Reynolds (1999)
Frieseomelitta varia	Insect	F	20	Captive	Nunes et al. (2008)
Synalpheus regalis	Shrimp	F	13	Captive	Duffy et al. (2002)
Neogobius melanostomus	Fish	N	14	Captive	Rollo and Higgs (2008)

Figure A4: Maximum effect size and 95% CIs calculated for studies published in six journals over the past decade. Also shown is the maximum effect documented in playback experiments with *Anolis gundlachi*, specifically that of response latency to the conspecific versus the nonconspecific stimuli. See figure 1 in the main text for other details.

Table A1

Latency of time *Anolis gundlachi* took to perform their first aggressive display to robot playbacks of conspecific and novel displays

	df	*F*	*P*
Overall model[a]	3, 169	2.87	.04
Independent variable:			
Intercept	1	196.83	<.001
Display type (F): conspecific vs. novel	1	.45	.50
Dewlap (F): present vs. absent	1	4.16	.04
Number of male neighbors (C)	1	5.84	.02

Note: Factors (F) and covariates (C) were analyzed in a univariate general linear model (ANCOVA). All covariates listed in table 1 and all covariate interactions with display type and dewlap factors were initially considered; they were eliminated from the model if $P > .10$. A display type × dewlap interaction was also initially included but was found not to be statistically significant at $P = .87$ and was removed. Four sets of playbacks were given: conspecific display with dewlap extended ($N = 50$), novel display with dewlap extended ($N = 46$), conspecific headbob display only ($N = 60$), and novel headbob display only ($N = 47$).

[a] FDR-$\alpha = .033$.

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