REVIEW

Deterring predators, daunting opponents or drawing partners? Signaling rates across diverse contexts in the lizard *Anolis sagrei*

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Abstract Although the visual display behavior in *Anolis* lizards has received ample attention, the function of dewlap extensions (DE), push-ups (PU), and head-nods (HN) in general, and in *Anolis sagrei* in particular, remains highly equivocal. Therefore, our primary goal was to quantify the display rates of these visual signal types (DE, PU, and HN) in a variety of contexts, using *A. sagrei* as study species. To our knowledge, this is the first study to test individuals of both sexes in a repeated-measures design across multiple contexts, including predator, non-predator and social interactions (mirror, male–male, male–female, female–male). We found that males have an overall higher signaling rate than females across all contexts. In addition, we found that lizards of both sexes exhibited higher display rates in the presence of conspecifics than when confronted with a predator or non-predator, suggesting that DE, PU, and HN function in intraspecific communication, not in predator deterrence. Whereas females did not significantly raise display rates in a consexual and heterosexual context with respect to subject-alone context, males did. The PU signal type only appears to play a major role for *A. sagrei* males during aggressive encounters. During heterosexual interactions, increased frequencies of all signal types suggest that DE, PU, and HN are essential for male courtship. Finally, we suggest that intersexual selection is probably a driving force for frequency-related dewlap use in both sexes. In contrast, pronounced intersexual differences were detected for PU and HN rates within a social context.

Keywords *Anolis sagrei* · Display behavior · Predator context · Social context

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Introduction

Animal communication is defined as the transfer of information between a sender and receiver via ritualized signals, involving a variety of sensory modalities (Bradbury and Vehrencamp 1998). Most animal groups focus primarily on a subset of possible modalities. Social insects, for instance, mainly use chemical signals, whereas anurans emphasize auditory signals (Gerhardt and Huber 2002). In the genus *Anolis*, a diverse group of neotropical, polychrotid iguanian lizards, visual displays are the primary mode of communication (Jenssen 1977; Carpenter 1978). These displays consist of conspicuous behaviors that are known to be used in multiple contexts, such as exhibiting territory ownership and territory defense (Greenberg and Noble 1944; Carpenter 1978), mate attraction and female receptivity (Greenberg and Noble 1944; Jenssen 1970a; Crews 1975; Sigmund 1983), species recognition (Rand and Williams 1970; Losos 1985), and predator deterrence (Leal and Rodriguez-Robles 1995, 1997; Leal 1999). The anole display repertoire typically consists of three major visual signal types that differ in their motion and conspicuousness: "head-nods" (up-and-down movement of the head), "push-ups" (up-and-down movement of the body and tail caused by flexion of the legs) and "dewlap extensions" (pulsing of the often-colorful throat fan or dewlap) (referred to by slightly different names in Scott 1984). Although each visual signal type can be displayed in isolation, it is frequently used in conjunction with other signal types, resulting in a stereotyped display pattern that tends to be species-specific, but nevertheless, shows considerable inter- and intraspecific variation (Jenssen 1977, 1978; Scott 1984; Fleishman 1992). It has been reported that all three signal types can appear across diverse contexts in both sexes of *Anolis* lizards (Jenssen 1970a, b; Hicks and Trivers 1983; DeCourcy and Jenssen 1994; Nunez et al. 1997). However, no study has

compared the respective display rates across a wide range of contexts using the same individuals. Also, previous studies have focused primarily on male signaling; female display behavior has received far less attention.

The brown anole (*Anolis sagrei*) has become a model for studying a variety of topics in ecology, animal behavior, and evolution. The species is native to Cuba and the Bahamas, but has successfully been introduced in many other areas (Campbell 1996). Males are polygynous, highly territorial, and substantially larger than females, but both sexes possess a bright yellow-to-reddish dewlap (Schoener and Schoener 1980; Schwartz and Henderson 1991). The general display repertoire includes dewlap extensions, push-ups and head-nods, but the frequency with which each signal type occurs varies considerably (Scott 1984; Partan et al. 2011). *A. sagrei* is also known to display readily under field and laboratory conditions, and across diverse social contexts (e.g., Scott 1984; Tokarz 2002; Tokarz et al. 2003, 2005; Simon 2007, 2011), which makes it an appropriate study species for examining display structure and behavior. Earlier studies on *A. sagrei* have primarily addressed the males' display behavior during territory defense and courtship (Scott 1984; McMann 2000; Paterson 2002; Tokarz 2002; Tokarz et al. 2003, 2005; Paterson and McMann 2004; Simon 2011), with equivocal results. Scott (1984) concluded that encounters with rival males and potential mates elicited similar dewlap extension rates in males, suggesting that the signal functions in both hetero- and consexual contexts. However, Tokarz et al. found that male *A. sagrei* with inoperative dewlaps mated at the same rate as normal males, suggesting that dewlap use is not necessary for obtaining matings (Tokarz 2002; Tokarz et al. 2005). In addition, they noted that males with inoperative dewlaps were just as likely to maintain their territories as normal males, indicating that dewlap signaling has no fundamental role in territory defense either (Tokarz et al. 2003). Simon (2011), on the other hand, found that males flashed their dewlaps more frequently during male–female than during male–male interactions, suggesting that dewlaps are used primarily in courtship. To our knowledge, only two studies have so far addressed the display rates of *A. sagrei* in response to predatory cues (Simon 2007; Elmasri et al. 2012). Although both studies used a similar predator (a kestrel model), Simon (2007) found a significant decrease in the signal rate of the high-amplitude push-ups after exposure to the visual predatory cues, whereas Elmasri et al. (2012) failed to find any change in display behavior of male *A. sagrei* to both visual and acoustic predatory cues. In short, the function of dewlap extensions, push-ups, and head-nods in the behavior of *Anolis* lizards in general, and *A. sagrei* in particular, remains highly equivocal. Therefore, our primary goal was to quantify the use of these visual signal types in a variety of contexts. In contrast to previous studies, we tested both sexes and used a repeated-measures design, exposing a large number of individuals to

diverse contexts, including predator (*Leiocephalus carinatus*), non-predator (*Uromastyx occelata*), and social interactions (mirror, male–male, male–female, female–male). All the encounters were staged in laboratory conditions. In this way, the same subjects could be tested repeatedly and in isolation across several contexts under fully controlled testing conditions. Rather than examining display structure, we focused on the frequency with which each individual signal type was performed; previous field studies have shown that predator and social context can affect the relative signal frequencies exhibited by *A. sagrei* (McMann 2000; Simon 2007, 2011). If the individual signal types serve an intraspecific communication function, we expect that lizards will primarily employ them in social contexts, including both hetero- and consexual interactions. In contrast, when they function in a predator deterrence communication system, we expect higher usage in the presence of a predator. We further predicted we would find a different role of the signal types in male and female *A. sagrei*, as selective pressures on signaling traits often differ between sexes (Blanco and De La Puente 2002; Vanhooydonck et al. 2005, 2009). Finally, we expected to improve our general understanding of the role of each visual signal type in *A. sagrei* by testing many individuals of both sexes.

Materials and methods

Animals

In this study, a total of 68 captive *A. sagrei* were tested, including 46 males and 22 females. All individuals were adults originally caught in Florida, and obtained via the pet trade (Fantasia Reptiles, Belgium, license HK51101419) in October 2011. The snout–vent length (SVL) of male and female individuals measured upon arrival ranged from 40.83 to 61.47 mm and 39.55–50.65 mm, respectively; the threshold size at which sexual maturation occurs in *A. sagrei* is considered 39 mm for males and 34 mm for females (Licht and Gorman 1970; Lee et al 1989). Outside experimentation, all anoles were housed individually in specially designed cages at the University of Antwerp, Belgium. Each cage (30 cm L× 22.5 cm W×27.5 cm H) contained a thick layer of peat bedding (Spaghnum, Novobalt) covered with banana tree leaf litter, a wooden perch (ca 40-cm length, 2.5-cm diameter) placed diagonally at a 40 ° angle, and a light source (Philips SpotOne 30 °, 25 W) set to a 12:12-h light–dark regime; cages had non-transparent walls to avoid any visual contact and social interactions among the anole lizards. The temperature within the cages ranged from 26 °C during dark regime to a max of 35 °C under the light source during light regime; the relative humidity of the animal room ranged from 55 to 70 %. All anole lizards had ad libitum access to water from a small

dish in their cage and were hand sprayed with water every other day. We fed each lizard every 2 days with 3- to 5-week-old crickets (*Acheta domestica*) and occasionally added wax moth larvae (*Galleria mellonella*) to their diet; both food types were dusted with a dietary supplement containing a 2:1 calcium/phosphor ratio and vitamin D3 (Zoo Med Reptivit with D3, Fantasia Reptiles, Belgium).

We additionally housed a male *L. carinatus* (curly tailed lizard) and *U. ocellata* (ocellated spiny-tailed lizard) during the course of the "predator" and "non-predator" experiments, respectively. Both animals were obtained via the pet trade, and kept for a maximum of 4 weeks (Fantasia Reptiles, Belgium, license HK51101419 and CITES 12NL120140/11). The two lizards were separately housed in a large terrarium (100 cm L×40 cm W×50 cm H) with appropriate environmental conditions, ad libitum water access, and the necessary dietary requirements (Schwartz and Henderson 1991; Schleich et al. 1996).

Experimental procedure

Male subjects were exposed to a total of five different experiments in the following order: (1) "predator (PR)," (2) "mirror (MI)," (3) "male–male interaction (MM)," (4) "non-predator (NP)," and (5) "male–female interaction (MF)" experiment. Females were subjected to four experiments: (1) PR, (2) MI, (3) "female–male interaction (FM)," and (4) NP. Experiments were generally conducted between March and September 2012, which is considered the breeding season for *A. sagrei*. Due to technical failure, one experiment (i.e., MF) was postponed and carried out between the second half of September and start of October 2012. All experiments were performed during the natural activity period of the anole lizards, i.e., between 0900 and 1600 hours. Staged encounters always involved interactions between unfamiliar subjects, as familiarity and prior physical contact can affect display and courtship behavior in the brown anole (Tokarz 2006; McMann and Patterson 2012). In principle, each individual was used only once per experiment (for exceptions: see section MM and MF experiment). Different experiments were carried out with a rest interval in between of at least 2 weeks to minimize disturbing the lizards and any carry-over effects. Only healthy individuals were selected for testing, resulting in a smaller sample size for experiments conducted at a later stage.

The following procedure was used in all experiments. A subject was taken from its home cage, and kept in an incubator for 40 min at 30 °C to ensure a similar body temperature in all lizards. Then, the lizard habituated to the experimental cage for a period ranging from 20 to 45 min. No measurements were carried out during this period. After habituation, the subject's display behavior was scored for the entire treatment duration. During all experiments, the temperature of the peat layer in the center of the terrarium was kept at 26±1°C, via an external electrical heater (SuperSer, model GL-15, 1,350 W). A high level of humidity (55–70 %) was maintained by hand spraying the terrarium before each habituation period. All sides of the experimental terrarium, except one (i.e., observer's side), were covered in white paper to prevent any distraction from the outside. The observer's side was transparent and enabled online behavioral scoring by an observer sitting still at a distance at least 2 m away from the terrarium. At the end of every experiment, cages were thoroughly cleaned.

Predator experiment

During the PR experiment, each subject was confronted with a single male curly tailed lizard. As *L. carinatus* is widely distributed in Florida and known to predate on brown anoles (Schoener et al. 2002), the species constitutes a good predator model for our experimental setup. The PR experiment was conducted in a large glass terrarium (100 cm L×40 cm W× 50 cm H) closed with a two-inlet lid on top which allowed some airflow. The terrarium was provided with a thick layer of peat substrate partly covered by a banana leaf and with a branch placed on top of the ground layer (Fig. 1a). Both the banana leaf and branch were taken from the home cages of the tested individuals. Two light sources (Philips SpotOne 90 °, 40 W) were mounted symmetrically on the inside of each inlet, i.e., left- and right-hand side of terrarium, providing an optimal temperature for testing. To confront our subjects with the predator, a small glass tank (20 cm L×20 cm W×20 cm H) containing the living *L. carinatus* individual was placed inside the large terrarium providing indirect contact between predator and prey. The "predator tank" containing the *L. carinatus* was fully transparent. Its base was fitted with a thin layer of peat and the top was covered with a mesh. Thus the set-up provided the visual, auditory, and chemical cues of a predator. During habituation, individuals were exposed to a "control tank" inside the large terrarium, i.e., an identical tank as described above, but without the predator (Fig. 1a). Then, the actual experiment started with two consecutive treatments per subject: a control treatment (C, with an empty tank) and a predator treatment (PR, with the predator in the tank). Each treatment lasted 20 min, resulting in a total behavioral observation time of 40 min per subject. During the whole experiment, subjects stayed inside the large terrarium and the appropriate tanks were changed via one of the upper inlets, with minimal disturbance. To rule out any confounding effects, control and predator tanks were placed alternatively on the left and right side of the terrarium with the same subject having the appropriate tank on the same side for both treatments. We followed a balanced, randomized test protocol to determine the position of the stimulus tank (i.e., left or right) and to define the order in which subjects were tested in the C and PR treatment. The *L. carinatus* individual behaved in a similarly passive way during all PR treatments thereby
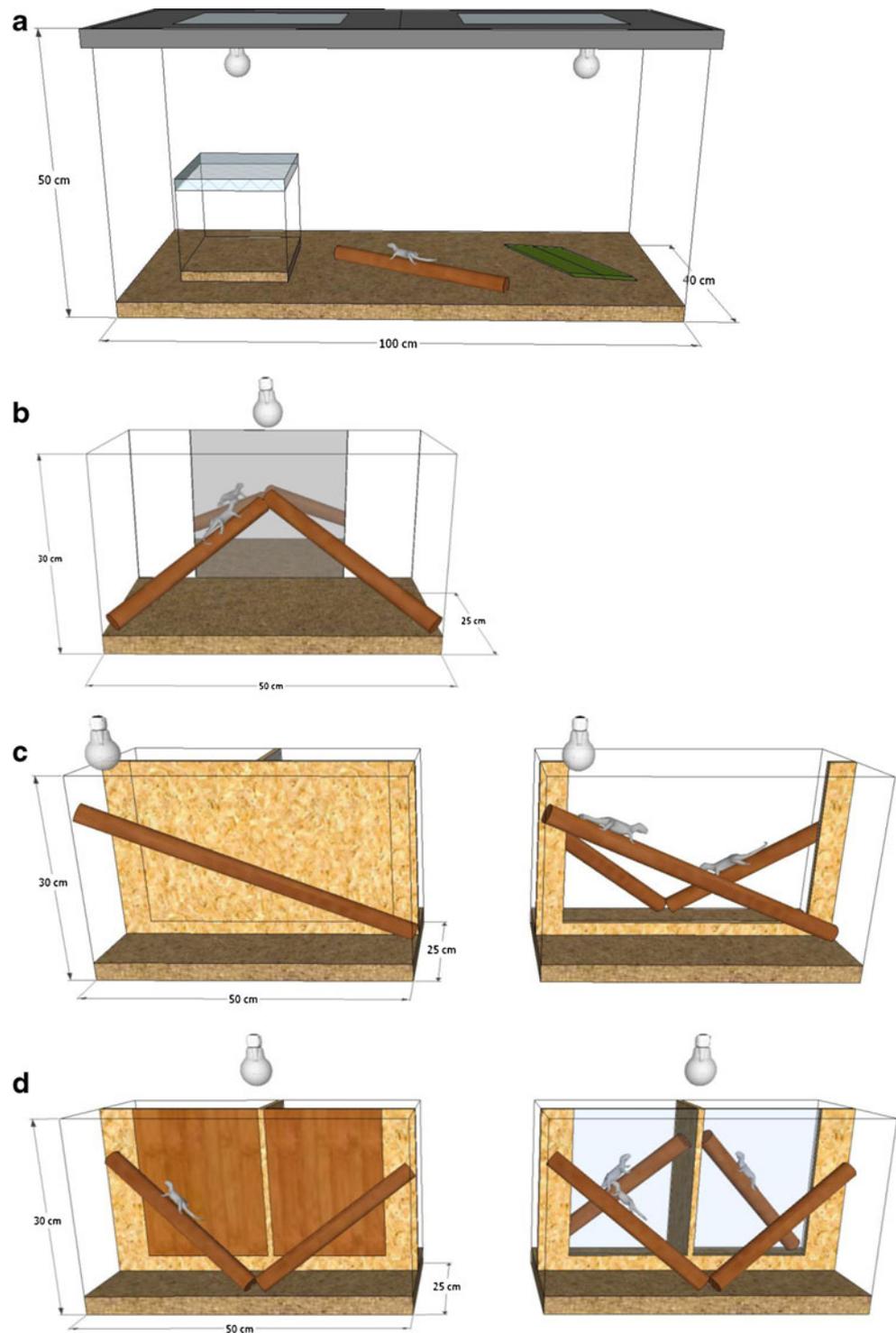
excluding possible effects caused by predator behavior and
movement.

Non-predator experiment

Set-up and design of the NP experiment were similar to that of
the PR experiment (Fig. 1a). However, instead of a predatory

lizard, an herbivorous heterospecific lizard was used.
U. ocellata inhabits rocky areas in the arid zones of northeast-
ern Africa (Schleich et al. 1996) and is, from its dietary
perspective (90 % herbivorous), harmless to our anole sub-
jects. The idea of incorporating the NP experiment was to test
whether the response to the *L. carinatus* subject during the PR
treatment is indeed a predator-specific response or rather a

generic response to any heterospecific subject of similar size. We selected a male *U. ocellata* with SVL and weight similar to that of the *L. carinatus* individual used in the PR experiment. As with the PR experiment, this experiment consisted of a control treatment (C, empty tank) and a non-predator treatment (NP, with the *U. ocellata* inside the tank). Choice of the position of the stimulus tank and treatment order was as in the PR experiment. The *U. ocellata* also behaved passively during all treatments, ruling out any possible effects of change in behavior and movement.

Mirror experiment

The MI experiment was conducted to investigate the display behavior of brown anoles when a subject is exposed to its mirror image, and thus to matching display behavior. For this experiment, a terrarium (50 cm L×25 cm W×30 cm H) was fitted with a peat layer and two equally sized branches positioned in a triangular way (Fig. 1b). A light source (Philips SpotOne 90 °, 40 W) was placed 20-cm above the intersection of the two branches (i.e., highest perch site) and created therefore an ideal common resource to compete for. A perforated wooden lid closed the terrarium. After habituation in the described set-up, a mirror (30 cm L×30 cm H) was placed against the exterior side of the terrarium wall (Fig. 1b). Next, one of us (TD) opened the wooden lid and, using a stick, gently directed the subject towards the mirror, to make it aware of the visual presence of a matching opponent. To avoid the possible effects of disturbance, observations of display behavior started only 2 min after the wooden lid had been closed. Observations in the MI treatment lasted 10 min per subject.

Male–male interaction experiment

For the MM interaction experiment, we matched 18 pairs of males for body mass and SVL because size affects dominance in anole lizards (Leuck 1995; Perry et al. 2004). The mean differences between members within pairs (± SE) were 0.26± 0.04 mm for SVL and 0.32±0.08 g for mass. To fulfill the matching requirements, five males had to be part of two different pairs. For those five males, the only data obtained during exposure to the first MM treatment were used in the analyses. For the MM experiment, a terrarium (50 cm L× 25 cm W×30 cm H) was divided into three compartments using a wooden panel; two equally sized smaller compartments at the rear and one larger compartment at the front (Fig. 1c). All compartments were provided with a peat layer and a diagonally positioned branch (rear compartments: branch of 25-cm length and 1.5-cm diameter; front compartment: branch of 55-cm length and 2.5-cm diameter). A light source (Philips SpotOne 30 °, 25 W) was placed above the end of the highest branch in the front compartment, creating a

resource for competitive interactions (Fig. 1c). Males were randomly assigned to one of the rear compartments and allowed to become accustomed to the new environment. We then removed the wooden panel, so that both males gained access to the larger front compartment containing the common resource. Observations started 5 min later and continued for 50 min. The display behavior of both males was assessed simultaneously. We were able to distinguish between individual males due to recognizable differences in color pattern and head shape. Display frequencies performed by matched males during the MM treatment, were not correlated within pairs (non-parametric Spearman's rho test per signal type, 2-tailed: all $p>0.6$).

Male–female interaction experiment

The experimental design and set-up of the MF experiment was analogous to that of the MM experiment (Fig. 1c), but involved the staging of 27 male–female couples. Couples consisted of a male and female subject that had no previous contact. As we retained a higher number of male subjects, some randomly selected females were assigned to two couples and thus were used more than once. We think this redundancy should not constitute a problem, as only the males' display behavior was scored in this experiment. Some couples copulated during the observation period ($N=7$ out of 27 couples). In these cases, any displays exhibited by the male during the copulation were disregarded. Tallying of the displays recommenced as soon as the animals stopped copulating. Data obtained before and after copulation were later pooled for those seven subject, as no significant difference was found for any signal type (non-parametric Wilcoxon signed rank test per signal type, before and after copulation, 2-tailed: all $p>0.4$).

Female–male interaction experiment

In the FM interaction experiment, we scored the display behavior of a female in the presence of two males located behind a transparent panel. The same size-matched pairs as for the MM treatment were used, and females were assigned to a certain pair based on their SVL, i.e., the largest females were confronted with the largest males. In all cases, females had never previously been in contact with either of the males. The set-up of the FM experiment resembled that of the MM and MF experiments, but after removal of the wooden panel, the rear and front compartments remained divided by a transparent Plexiglas sheet (Fig. 1d). As a consequence, the female in the front compartment was able to see the males in the rear compartments and vice versa, but could not reach them. The males in the rear compartments could not see each other. In each rear compartment, a branch (25-cm length, 2-cm diameter) was diagonally positioned at a 45 ° angle with the highest branch end pointing to the terrarium center. In the front

compartment, two equally sized branches (30-cm length, 2.5-cm diameter) were positioned in a V-shape, leaning against the terrarium at the observer's side. A light bulb was positioned 10 cm above the terrarium center (Philips SpotOne 30 °, 25 W). Only the display behavior of the female subject was observed for a total amount of 50 min, consisting of five 10-min observation bouts with a 1-h interval between observation sets. During all trials, at least one of the male subjects showed display behavior towards the female.

Data acquisition and behavioral analysis

An observer was present to score the subjects' display behavior online during all trials. We additionally made camera recordings (Sony handycam HDRCX 260) to facilitate offline analysis at a later stage, in cases of unclear online observations. All the observations were scored and analyzed using JWatcher event recorder software (version 1.0; Blumstein et al. 2000). We distinguished between three visual signal types: dewlap extensions (DE), push-ups (PU), and head-nods (HN). A dewlap extension was defined as one complete extension and retraction of the dewlap, a push-up as one single up and down movement of the whole body caused by flexion of only the front legs or all four legs, and a head-nod as one single up and down movement that involved only the head. For each subject, the number of executed displays was counted during the described treatments and expressed as a frequency variable (counts/minute). We inspected plots of display frequencies against observation time (averages per sex) for all experiments and found no obvious trends in time. Therefore, we could standardize the obtained counts over time to enable us to compare the signal type variables among treatments with different durations. Treatments involving the interaction of conspecifics (i.e., MI, MM, MF, and FM) are considered as "social context" experiments within this study, whereas the PR and NP treatments are categorized under "predator context." The C treatment, which can be regarded as "subject-alone context" characterized by the advertisement of undirected signals, serves to assess a general baseline of the subject's display behavior.

Statistical analyses

We used generalized estimating equations (GEE) with repeated measures to test for the effect of experimental treatment on display frequencies. We assumed that each display variable (DE, PU, HN) followed a Tweedie distribution and used a log link as model type. We further assumed an independent working correlation matrix, which is recommended when prior information about the correlation between measurements of the same individual is lacking (Pan 2002). GEE analyses were run separately for each sex and display variable, with

"treatment" taken as predictor variable (C, PR, NP, MI, MM, and MF for males; C, PR, NP, MI, and FM for females) and DE, PU, and HN as dependent response variables. The obtained GEE parameter estimates were always calculated with respect to the C treatment. We decided only to include data of the first C treatment (PR experiment), because C treatments of PR and NP experiment did not differ significantly for any signal type variable (non-parametric Wilcoxon signed rank test for both sexes separately, 2-tailed, all $p>0.1$). Differences in signaling frequencies between any two treatments were examined using pairwise comparisons of estimated marginal means, applying sequential Bonferroni adjustments to correct p values for multiple testing. Statistical analyses were conducted with the statistical software package SPSS version 20 (SPSS, Chicago, IL, USA) and a value of $p<0.05$ was considered as statistically significant.

Results

Even though statistical analyses were run separately for sex, we still provide descriptive information regarding the magnitude of the signal type frequencies in males versus females across diverse contexts (Fig. 2). Overall, males signal at higher rates than females, with a distinct difference being found for the DE signal type. Males tend to dewlap much more than females across all contexts (second order mean ± SE for DE frequency including all treatments: 1.14±0.23 cpm in males versus 0.07±0.04 cpm in females). Males perform more PU than females in both consexual and heterosexual interactions (second order mean ± SE for PU frequency including all treatments: 1.42±0.62 cpm in males versus 0.25± 0.09 cpm in females). The sexes differ less in their HN frequency (second order mean ± SE for HN frequency including all treatments: 0.54±0.11 cpm in males versus 0.31± 0.12 cpm in females).

The effect of treatment on signaling frequency in males was highly significant for each individual signal type (GEE, DE [Wald χ^2=27.78], PU [Wald χ^2=41.81], HN [Wald χ^2= 24.21], all $p<0.001$). We observed an overall lower signaling rate in males for all signal types in the "predator context," i.e., both PR and NP treatment (Fig. 3a–c). However, a statistically significant decrease in frequency between the predator (PR and NP) and social context treatments (MI, MM, and MF) was only found for the PU signal type. When confronted with male stimuli (MI and MM), males performed more PU, but not DE and HN compared to subject-alone and predator context (Fig. 3a–c). Last, males increased their DE, PU, and HN frequencies simultaneously when exposed to cues of conspecific females (MF) (Fig. 3a–c).

In females, treatment had a strong effect on signaling frequency for the PU and HN, but not the DE signal type (GEE, DE [Wald χ^2= 8.38, p=0.079]; PU [Wald χ^2=14.16,
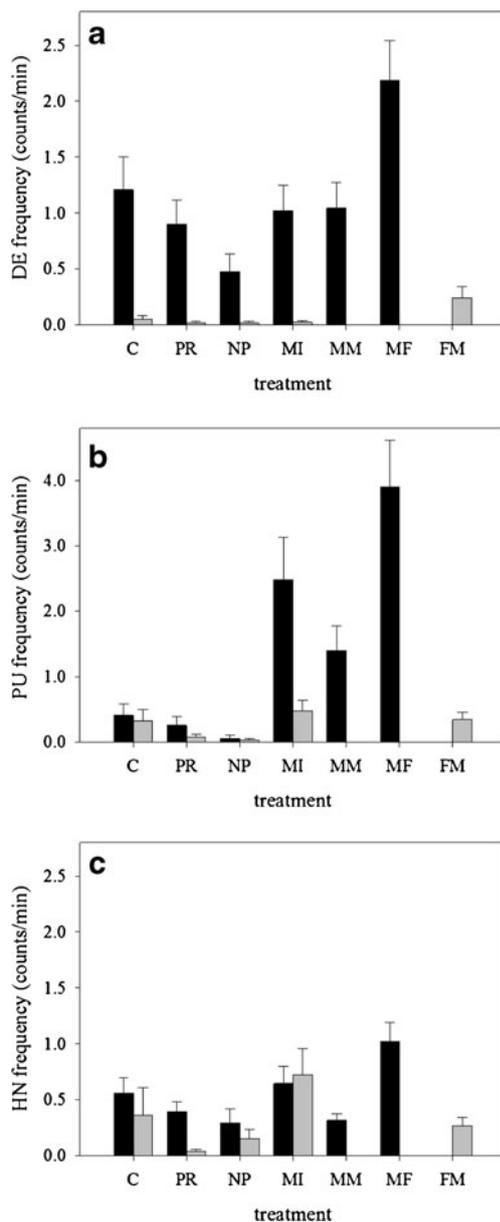
Fig. 2 Signal type frequencies across diverse treatments, in males versus females. Males (*black bars*) were exposed to a total of six treatments (*C, PR, NP, MI, MM, MF*) and females (*gray bars*) to five (*C, PR, NP, MI, FM*). Each signal type is represented in a separate panel: **a** Dewlap extensions (DE), **b** Push-ups (PU), and **c** Head-nods (HN). In all panels, first order means and SE are shown. Treatment abbreviations: *C* control, *PR* predator, *NP* non-predator, *MI* mirror, *MM* male–male interaction, *MF* male–female interaction, and *FM* female–male interaction treatment. Sample size per treatment and sex (*M* male, *F* female): *C* and *PR*, N=46 *M* and 22 *F*; *NP*, N=30 *M* and 16 *F*; *MI*, N=36 *M* and 21 *F*; *MM*, N=31 *M*; *MF*, N=27 *M*; *FM*, N=17 *F*

p=0.007]; HN [Wald χ^2=15.31, p=0.004]). Like males, females decreased their overall display frequency in the presence of a predator and non-predator compared to the subject-alone and social context (Fig. 3d–f). Here, the decrease was most pronounced for PU during the NP treatment and HN

during the PR treatment (Fig. 3e and f). With regard to the consexual context (MI), females showed no clear changes in behavior in response to their mirror image (Fig. 3d–f). Last, when confronted with conspecific males (FM), no significant increase in display rate was found for any of the signal types (Fig. 3d–f). However, there was a strong trend showing that females performed more DE in the heterosexual context compared to all other treatments (all *p*<0.05 without sequential Bonferroni correction) (Fig. 3d).

Discussion

Signaling in a predator context

When confronted to the predatory cues of a living curly tailed lizard (*L. carinatus*), both sexes showed an overall decrease in the signaling rate of all three signal types in comparison to the subject-alone and social context. The drop in signaling frequency was particularly prominent for PU in males and HN in females. In a field study, Simon (2007) demonstrated that male *A. sagrei* individuals marginally decrease their DE rate and exhibit significantly less PU in response to a simulated predatory attack by a kestrel model (PU were defined as "four-legged bobbing displays"). Two other recent studies on *A. sagrei* found no effect of imminent predation on display rates and times (Elmasri et al. 2012: visual and acoustic cues of bird predators; Yee et al. 2013: rubber snake model). In conclusion, our results and those of previous studies on a variety of predators (Simon 2007; Elmasri et al. 2012; Yee et al. 2013) strongly suggest that DE, PU, and HN do not function as a pursuit-deterrence signal (i.e., prey communicate their vigilance to a predator, thereby discouraging it from attacking; Hasson 1991). This conclusion is in contrast with findings by Leal and Rodriguez-Robles (1995), who demonstrated that in *Anolis cristatellus*, several signals used during social interactions (e.g., number of DE and PU; the latter defined as "four-legged bobbing displays"), were also used as deterrent signals in response to a natural predatory snake. It follows that the use and role of display signal types can differ strongly among closely related species. Vanhooydonck et al. (2009) found that *A. sagrei* males and females tend to have larger dewlaps on islands where *L. carinatus* occurs in sympatry, and suggested that the dewlap serves a pursuit-deterrence role. As we observed an opposite result for dewlap use in a predator context with *L. carinatus*, we conclude that selective pressures acting on dewlap size and use can act in a different way.

In an attempt to complement those previous studies on *A. sagrei*, we incorporated a NP treatment to enable us to test for a predator-specific response rather than a general response to any kind of heterospecific lizard. As our subjects reacted in a similar way during the PR and NP treatment, we could not find evidence for a predator-specific
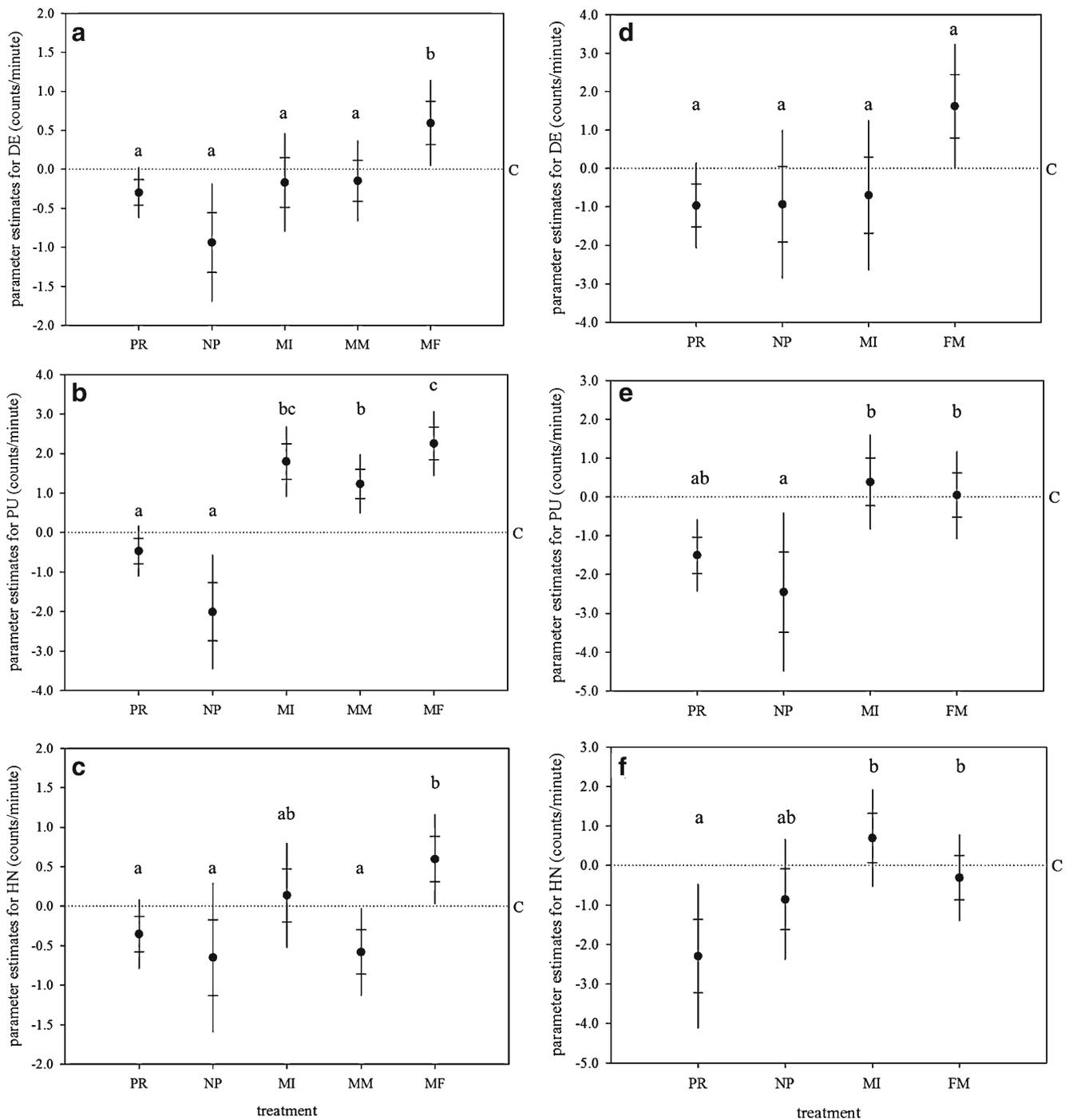
Fig. 3 GEE parameter estimates for signal type frequencies in *Anolis sagrei*, shown for multiple treatments with respect to control treatment (*C*, *dotted line*). Columns of panels represent different sexes: **a**–**c** results for males and **d**–**f** results for females. Rows of panels represent individual signal types: **a**, **d** Dewlap extensions (DE), **b**, **e** Push-ups (PU), and **c**, **f** Head-nods (HN). Separate GEE analyses were run per sex and signal type testing treatment main effects; displayed values are mean (*black dot*), SE and 95 % CI. *Different letters* denote significant differences between treatments with *p*<0.05 (pairwise contrasts between estimated marginal means adjusted for multiple comparisons using sequential Bonferroni). For treatment abbreviations and sample size, see legend Fig. 2

response towards the curly tailed lizard. The observed reduction in signaling rate during the PR treatment can thus either be explained in a risky predator context where minimum visibility would be beneficial to avoid predators

(Stuart-Fox et al. 2003) or in a harmless heterospecific context where signaling to inappropriate mates and opponents would result in unnecessary energy expenditure (Andersson 1994).

Male signaling in a social context

We examined male signaling in a consexual context in two types of experiments. In the MI experiment, males were exposed to their mirror images (only visual cues) and in the MM experiment, males were exposed to real size-matched male opponents. We found overall similar display rates (DE, PU, HN) for both consexual experiments, which confirms the earlier finding that *Anolis* lizards use visual rather than chemical signals during social interactions (Jenssen 1977; Fleishman 1992; Jenssen et al. 1995; Forster et al. 2005). We further observed that *A. sagrei* males increased their PU frequency, but not their DE and HN rate during staged consexual interactions. The role of DE and HN in male aggressive encounters seems thus limited in *A. sagrei*. Analogous to our DE findings, Simon (2011) showed that DE rates in male *A. sagrei* were lower during agonistic encounters than during courtship. Besides, Tokarz et al. (2003) revealed that males with inoperative dewlaps show the same ability to defend territories as normal males. Possibly, the benefits of dewlap use in a consexual context do not outweigh the potential predation cost associated with its use, as higher DE rates correspond to higher conspicuousness. Also, DE displays could be risky in a male–male context, because dewlaps are thin flaps of skin that could easily be damaged during escalated fights (suggested by Decourcy and Jenssen 1994). The PU signal type, on the other hand, seems to be of high importance during our staged male encounters conducted under laboratory conditions. Previous field studies have already shown that male *A. sagrei* produce higher bobbing rates ("bobbing" corresponds to PU following our terminology) in situations that may increase aggressive arousal, e.g., territory establishment and defense, when intruders are within close proximity, and when meeting unfamiliar rivals (e.g., McMann 2000; Paterson and McMann 2004; Simon 2011). Moreover, in *A. cristatellus* males, a positive correlation has been demonstrated between the number of bobbing displays and endurance capacity in a context of territorial defense and predator avoidance, suggesting that PU serves as an honest signal of endurance (Leal 1999; Perry et al. 2004). Thus, males may be able to assess the endurance capacity of opponents, via their exhibited PU rates.

In the heterosexual context, tested by the exposure to an unfamiliar female, males showed higher signaling rates for all three signal types (DE, PU, HN). In accordance with our laboratory results for the DE signal type, Simon (2011) found increased DE rates for male *A. sagrei* during heterosexual interactions under natural conditions, suggesting that dewlap use indeed plays an important role in male courtship. However, the effect of DE on courtship success might be relatively subtle because several earlier studies found no significant differences in mating frequencies between males that were experimentally prevented from extending the dewlap

and sham-treated control males (Tokarz 2002; Tokarz et al. 2005). Perhaps male DE functions in sex recognition and/or stimulate female receptivity (Crews 1975; Stamps 1977; Orrell and Jenssen 2002; Simon 2011), but additional research is necessary to further investigate these proposed functions. Also, the high PU frequencies we observed in males during heterosexual interactions are in line with Simon's (2011) finding that high bobbing rates ("bobbing" corresponds to PU following our terminology) are associated with successful mating encounters. Although the PU signal type is considered to be an honest signal for endurance in a territorial and predator context (Leal 1999; Perry et al. 2004), it is unknown if this acts in a similar way during courtship. There is no convincing evidence that female anoles make comparisons between males in making their mating choices (Tokarz 1998; Jenssen et al. 2001) and therefore, it is questionable whether high signal rates possibly associated with male quality will be advantageous during courtship. Similar to the DE signal type, HN as individual signal type seems to be extensively used during courtship only, and may, therefore, serve a particular function in this context. Finally, it should be noted that a simultaneous increase in all three signal types was seen only in the heterosexual context. By combining extensive signaling rates involving DE, PU, and HN, displaying males appear more conspicuous during courtship than in any other context. Perhaps, the conspicuous male displays may advertise sexual receptivity to the female and act as a motivational component for copulation.

Female signaling in a social context

In the consexual context, female individuals did not explicitly increase any display rates in response to their mirror image. This suggests that none of the tested signaling types play an essential role during territorial and agonistic encounters in *A. sagrei* females. Previous studies on other anole species have, however, shown that similar to males, females often defend exclusive territories (e.g., Rand 1967; Jenssen 1970a; Nunez et al. 1997; Orrell and Jenssen 1998). They may additionally benefit from signaling aggressively to other females by obtaining control over particular resources, such as food and nesting sites (West-Eberhard 1983). Yet, two studies on *Anolis carolinensis* have reported that consexual competition for resources has minimal effects on the female potential reproductive rates, indicating that the use of aggressive signals among females should be under minimal intrasexual selection (Nunez et al. 1997; Jenssen et al. 2000). With respect to our findings, it should be noted that we confronted females only with their mirror image and not with real adversaries presenting both visual and chemical cues. Besides, we used a similar resource to compete for (i.e., light source and highest perch site) in both sexes. Future work involving observations of real female encounters competing for other resources such as

nesting sites and food is required to further clarify the female display behavior in a consexual context.

Also, in the heterosexual context, females did not significantly change display frequencies compared to most other treatments. However, the strong increasing trend found for female dewlap use in the presence of a male cannot be ignored. Increased dewlap rates in females may be of importance in a context of male mating choice. Male preference for showy females has previously been demonstrated in guppies (Amundsen and Forsgren 2001) and some bird species (Amundsen 2000), but to our knowledge has not been reported in lizards. Besides, conspicuous DE exhibited by the female may also advertise sexual receptivity to males, prior to copulation. Sexual coercion imposed by males might be costly to both individuals and may additionally reduce female fecundity and survival (Clutton-Brock and Parker 1995; Le Galliard et al. 2005); advertising sexual receptivity may thus be beneficial for females.

Male versus female signaling

Generally, males signal (DE, PU, HN) at higher rates than females across all contexts, with a more pronounced intersexual difference being found for the DE signal type. This result is in accordance with previous studies on anoles, addressing male and female DE rates in subject-alone and differential social contexts on the field and under laboratory conditions (*A. carolinensis*: Jenssen et al. 2000; Orrell and Jenssen 2003; *A. sagrei*: Partan et al. 2011). For the overall PU and HN rates in males versus females, inconsistent use of terminology (see Partan et al. 2011 for more details) and a lack of data on those two signal types in females, hamper a comparison with other studies.

We further found that both male and female subjects exhibited higher display rates in the presence of conspecifics than when confronted with a predator or unknown heterospecific, indicating that DE, PU, and HN function in intraspecific communication for both sexes, and not in predator deterrence. Within a social context, displaying of the dewlap in *A. sagrei* seems to play an essential role during the male courtship of females only. However, we found a strong trend that *A. sagrei* females use their dewlap far more in the presence of a male, suggesting that selective pressures on dewlap use act in a similar way for both sexes. Unlike dewlap displays, we found pronounced intersexual differences in the PU and HN use during social interactions. The PU signal type seems to be an essential key factor during aggressive encounters in males, but not in females. And increased HN rates were only found in the male sex during heterosexual interactions, indicating an important contribution of HN during male courtship. From these results, we suggest that within a social context, selective pressures acting on the PU and HN signal type differ between sexes.

We end with some cautionary notes on the generality of our findings. First of all, we only consider frequencies of three main individual signal types in our study. Even though a higher frequency of a certain signal type would indicate more functionality of that particular signal type within a certain context, rates of various display types including the combination of a particular set of signal types might also serve a crucial role in the respective contexts. Also, additional studies are required to assess the importance of more signal type parameters (e.g., speed, duration and absolute amplitude) and other signal types (e.g., crest erection, licking, yawning…) in the display behavior of *A. sagrei*. Second, our study was conducted under fully controlled laboratory conditions and subjects were tested separately and repeatedly. Although our results generally accord with findings from field studies, we admit the difficulty of correctly interpreting display behavior in nature on the basis of laboratory results. Third, all our experiments were tested in standardized lab terraria, providing only a framework for short-distance interactions. Earlier studies have shown that short versus long distances can affect the signaling behavior across diverse contexts (Hasson et al. 1989; Decourcy and Jenssen 1994; McMann 2000), indicating that "distance" should be accounted for in the general interpretation of the *A. sagrei* display behavior.

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