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## Persistence of individually distinctive display patterns in fatigued side-blotched lizards (*Uta stansburiana*)

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**Abstract** Iguanian lizards perform conspicuous species-typical push-up displays, which are used in territory advertisement, threat, and courtship contexts. Subtle individually distinctive differences in push-up characteristics have been suggested to play a role in the recognition of social partners. However, if the structure of push-ups is responsive to changing physiological states then their capacity to promote recognition may be limited. The current study evaluated whether the push-ups performed while in an experimentally imposed state of fatigue by male side-blotched lizards (*Uta stansburiana*), retain the individually distinctive characteristics apparent in rested lizards. We found repeatable among-individual differences in the duration and the relative height of push-up components. Repeatability values did not change consistently between the rested and fatigued conditions, nor when both conditions were pooled, indicating that these push-up characteristics do not change with fatigue. Similarly, discriminant functions that were generated using push-ups from one state assigned push-ups performed in an alternate state to the correct individuals. Furthermore, when analyzed independently of individual identity, the values of display parameters examined in the current study did not change significantly between states, and discriminant function analysis could not reliably classify push-ups to the correct state. Taken together the results show that individually distinctive push-up characteristics are robust to effects of fatigue, consistent with

their suggested role in social recognition. In the future, video playback experiments can test whether lizards utilize the signature-like characteristics of push-ups to discriminate among individuals.

**Keywords** Visual communication · Individual recognition · Push-up display · Lizard · *Uta stansburiana*

### Introduction

For a diversity of animals, the recognition of social partners by means of distinctive chemical, visual and vocal signatures is fundamental to the maintenance of social relationships (Colgan 1983). In relationships such as dominance, territoriality, mating, and parenting, recognition allows appropriate behaviors to be directed towards specific social partners (Wilson 1975; Falls 1982). Territorial neighbors in many taxa develop ‘dear enemy’ relationships, which reduce the costs of territory defense (Ydenberg et al. 1988). Neighbors appearing at the shared territorial boundary pose a low risk of territory takeover and are tolerated, while aggressive attacks are directed at individuals that pose a significant risk of territory takeover, including neighbors opposite of their normal location and strangers at any location (Brooks and Falls 1975; Falls and Brooks 1975; Davis 1987). Playbacks of vocal signatures are sufficient to elicit these differential responses, indicating the importance of signal variation in mediating the recognition of social partners (reviewed in Falls 1982; Ydenberg et al. 1988).

Iguanian lizard perform stereotyped species-typical visual displays in which the head, or the head along with the entire trunk, progresses through a series of rapid up-and-down movements (Carpenter and Grubitz 1960; Carpenter and Ferguson 1977). These ‘push-up’ displays may be performed in a variety of contexts, including courtship, aggression and territory advertisement, have been suggested to have several functions (Martins 1993), and vary at several levels (Jenssen 1978). Push-ups vary among species and appear to play a role in species

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recognition (Carpenter 1962; Hunsaker 1962; Ferguson 1973). Varying degrees of population differentiation have been described within some species (e.g., Ferguson 1970; Jenssen 1971; McKinney 1971b; Martins et al. 1998). Within individuals, postural modifiers, variation in intensity and the use of discrete types are apparent in push-up displays, and may function in advertising levels of motivation and aggressive intent (Carpenter 1967; Hover and Jenssen 1976; Jenssen 1979; Martins 1993). Push-up frequencies and the duration of the associated laterally compressed threat posture are correlated with locomotor endurance, serving as an honest signal of fighting prowess to conspecific rivals (Brandt 2003) and to predators (Leal 1999). Push-up displays can be distinctive at the level of the individual, and thus have the potential to mediate the recognition of social partners (e.g., Jenssen 1971; Stamps and Barlow 1973; Berry 1974; Crews 1975; Rothblum and Jenssen 1978; Dugan 1982; Martins 1991). Lizards are known to discriminate between familiar and novel rivals (Glinski and Krekorian 1985; Qualls and Jaeger 1991; Fox and Baird 1992; Olsson 1994; Whiting 1999; Lopez and Martin 2001), and can also distinguish among territorial neighbors (Husak and Fox 2003).

To effectively mediate the recognition of individuals, a signal must be individually distinctive, and signal characteristics must be resistant to a spectrum of commonly experienced signaling conditions and physiological states. Possessing low aerobic capacity, many Iguanian lizards support even moderate levels of activity anaerobically (Bennett 1982), resulting in the accumulation of lactate, an agent of muscle fatigue (Fitts 1994). Lizards engaged in routine activities in the field sustain levels of lactate that are considerably elevated over baseline (Bennett et al. 1981; Pough and Andrews 1985a, 1985b), and hence can be expected to experience moderate levels of fatigue on a regular basis. Because fatigue modifies the contractile characteristics of skeletal muscle (MacIntosh and Allen 2000), we surmised that fatigue can affect push-up structure, thereby limiting the capacity of push-ups to advertise individual identity. Moreover, if fatigue exerts a consistent effect on push-up structure, then push-ups may reveal the state of the signaler. In contrast, if individually distinctive characteristics are resistant to the effects of fatigue, then the potential of push-up structure to promote recognition is preserved, whilst the capacity of push-up structure to reveal fatigue may be limited.

The present study contrasts the capacity of push-ups to serve as a template for individual recognition against the potential of variation in push-up structure to reveal a state of fatigue, by statistically comparing the structure of push-ups performed in a rested state to push-ups performed in an experimentally induced state of fatigue. We first pooled both states to obtain a large sample for determining repeatability values of a series of duration and relative height parameters. We then incorporated these parameters into a discriminant function analysis (DFA), using the rate of correct assignment as a measure of individual distinctiveness. By randomly sub-sampling

from our data set, we evaluated the effect of varying the number of individuals and the number of push-ups included in DFAs on our measure of individual distinctiveness. In the main analysis we compared repeatability values between rested and fatigued push-ups, and used DFA to evaluate whether the structure of push-ups performed in one state could be used to assign push-ups performed on the alternate state to the correct individual. For completeness, we tested whether the structure of push-ups performed in the fatigued condition differ consistently from push-ups performed in the rested condition, independent of individual identity.

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## Methods

### Subjects

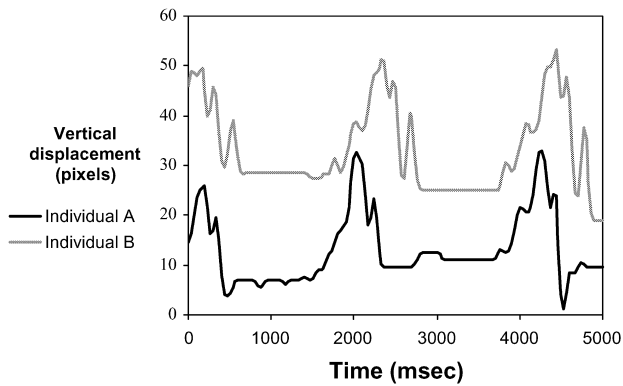
Adult male side-blotched lizards (*Uta stansburiana*,  $n=24$ ) were collected in April 1997, the annual peak of reproductive activity, at Los Banos Grandes, Merced Co, Calif. (California Department of Game and Fish permit no. 4745 to Barry Sinervo). Males and females of this small (5–10 g) insectivorous species perform one basic type of push-up in all contexts, although males perform at much higher rates than females (Ferguson 1970). Push-ups are performed singly, as lizards move about their home range, or in series, when another lizard is encountered. Variation in push-up structure has been reported between populations of this species, and in some cases intra-population push-up structure polymorphism was also noted (Ferguson 1970; McKinney 1971a, 1971b).

Subjects were maintained indoors, in terraria with a sandy substrate and a cardboard shelter. Light and a thermal gradient were provided in each terrarium by an incandescent lamp. Lights were maintained on a 13:11 light/dark schedule, with light onset at 7 a.m.. Subjects were offered crickets *ad libitum*, and water was provided by misting the inner wall of terraria. The entire experiment was conducted within 3 days of collecting the subjects.

### Experimental design

To examine the effect of fatigue on individual differences in push-up characteristics, we videotaped the behavior of each subject in a rested state and in a fatigued state. For the rested state we took subjects directly from their home cage and filmed their behavior for 5 min in an experimental arena (details below). Subjects were then induced to run on a motorized treadmill to obtain an index of endurance for each individual (details below). On the following day push-ups performed in a fatigued state were videotaped, using subjects that were first exercised on a treadmill for one half of their endurance capacity.

To obtain push-up sequences for analysis, subjects were placed individually in an experimental arena 20×40×20 cm (width×length×height), with a substrate of washed play sand. An incandescent light was placed over this arena to create thermal conditions that allowed subjects to maintain field active temperatures (35–38°C). To elicit push-ups, a mirror was situated along one side of the arena. Three sides of the arena were surfaced with cardboard to minimize extraneous reflections, while the fourth side was left uncovered to allow for filming the trial. At the start of the trial a subject was placed in the center of the arena, parallel to the mirror and perpendicular to the filming trajectory. Trials were filmed from the shelter of a blind (Sugerman and Hacker 1980) using a Hi8 video camera at 30 frames/s, with each trial lasting 5 min. Trials were conducted between 10 a.m. and 4 p.m., the primary hours of activity at this time of year (personal observation). The endurance of each subject was determined by the elapsed time to reach exhaustion while running on a motorized treadmill,



**Fig. 1** Time-motion analysis of the push-up displays of two male side-blotched lizards (*Uta stansburiana*). The vertical location of the eye is plotted as a function of time (in ms). Shown is a 5-s time interval, in which approximately three push-ups were performed by each individual

advancing at 0.5 km/h, a speed commensurate with that of males patrolling their territories in the field (Sinervo et al. 2000). Lizards were motivated to run by tapping on the hind legs and on the base of the tail, and exhaustion was determined by loss of the righting response (Huey et al. 1984).

#### Analysis of the visual signal

##### Data acquisition

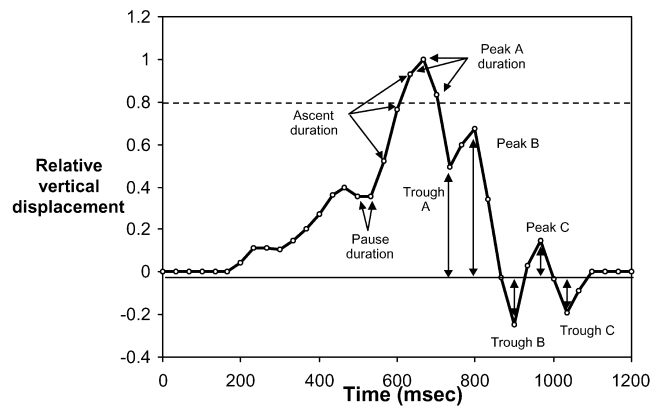
We converted short segments of video into display action patterns, which plot the vertical displacement of the head over time (Carpenter and Grubitz 1960). Selected segments of analog Hi8 video, filmed at a rate of 30 frames/s, were captured and converted to digital data using Macintosh iMovie software through a Sony i.Link digital AV interface. Digital video clips were then converted to Quicktime format and imported into NIH Image video analysis software for frame-by-frame analysis. We used a custom-programmed NIH Image macro, which allowed us to track and record the position of the subject's eye relative to its initial position.

##### The unit of display

Push-ups are executed either singly or in a series (Fig. 1), with each push-up lasting approximately 1 s, and successive push-ups in a series being separated by intervals lasting approximately 1 s. The push-up displays of individual lizards in this population exhibited observable differences (e.g., Fig. 1). To quantify individual variation in push-up characteristics, we adopted the approach employed by previous analyses of push-up structure in this species, treating each push-up as a unit of display (Ferguson 1970; McKinney 1971a, 1971b). Our analysis was designed to test whether temporal and relative height parameters of the elements within a single push-up are individually distinctive and whether individual differences observed in the rested state are carried over to the fatigued state.

##### Processing the push-up display action pattern

The push-ups of side-blotched lizards are extremely jagged, making the consistent identification of display sub-units and the quantification of display parameters difficult. We therefore smoothed each DAP plot by transforming each vertical displacement data point with a weighted moving average of immediately adjacent points using the following equation:  $[(\text{previous frame}) + 2 \times (\text{focal frame}) +$



**Fig. 2** A plot of a single push-up display illustrating the variables used to describe push-up structure in the present study. Each point along the plot represents the vertical position of the head at a specified frame of film (30 frames/s). The maximum vertical displacement relative to the resting position was labeled peak A, and its vertical displacement was standardized to one unit. Three duration parameters were obtained by counting frames (*single-headed arrows*: pause duration, ascent duration, peak duration; see the text for further definitions). Five relative vertical displacement parameters were obtained (depicted as *vertical double-headed arrows*: trough A, peak B, trough B, peak C, trough C)

(next frame)] $\times 0.25$ . Smoothing may obscure some individually distinctive characteristics. To make the push-ups directly comparable across individuals and signaling episodes we standardized push-up height by dividing each vertical displacement value by the maximum vertical displacement observed in that push-up. This manipulation set the peak height of each push-up to equal one unit, and all other amplitude parameters were computed in proportion to this unit height (Fig. 2). Standardizing push-up height eliminated any variation in amplitude due to differences in the distance between camera and subject, but also eliminated intrinsic variation in amplitude, an attribute that may be individually distinctive (e.g., Dugan 1982). Due to the distance confound, we could not quantify absolute variation in push-up amplitude. Smoothing and standardizing the amplitude of push-ups makes our analysis of individual distinctiveness conservative.

##### Display parameters

The maximum vertical displacement of each display defined the location of peak A, and subsequent maxima and minima were successively labeled B and C. The relative amplitude of these features provided five of the push-up parameters: trough A, peak B, trough B, peak C, trough C (Fig. 2). Some push-ups lacked distinct peak C and trough C. In these cases we coded the relative height of peak C and trough C as equaling that of trough B. In addition to the relative amplitude parameters, we defined three duration parameters, quantified by counting frames of film (Fig. 2). Pause duration is the interval between the minor peak that precedes peak A and the start of the ascent to peak A (where the slope becomes positive again). Ascent duration is the number of frames from the start of the ascent to peak A, to the highest point in peak A. Peak A duration is the number of frames in which the relative displacement of peak A remained above 0.8. While these parameters do not completely describe all details of push-up structure, they describe the most prominent elements of the push-up display and characterize much of the observed variation.

### *Selection of individuals and push-ups for analysis*

Of 24 subjects sampled, we selected the 12 individuals with the largest number of push-ups, for statistical analysis; each of these individuals performed at least 11 push-ups. The push-up sample for each individual came from at least two separate series of push-ups. To provide a balanced set of data in our first series of analyses, we included exactly 11 push-ups for each of these 12 individuals. The main series of analyses, which examined the stability of push-up structure between states, utilized eight individuals that performed multiple push-ups in both states.

### *Statistical analysis*

#### *Software used and repeatability analysis*

We used SPSS version 10 software for all of the statistical analyses, along with randomization and re-sampling procedures implemented using Resampling Stats (Bruce 2002). Analysis of variance (ANOVA) was used to test whether push-up parameters varied significantly between individuals. For each push-up parameter we calculated repeatability, the proportion of the total variation in a sample that is attributable to differences between individuals, rather than variation within individuals (Lessells and Boag 1987). Also known as the intra-class correlation coefficient, repeatability can be obtained from a one-way ANOVA as  $s^2_A/(s^2_A+s^2_W)$ , where  $s^2_A$  is the among-individual variance component and  $s^2_W$  is the within-individual variance component.

High repeatability values of push-up parameters are necessary but not sufficient to establish individual distinctiveness. High repeatabilities can result when individuals have a narrow range of values for each parameter compared to the group at large, while overlapping extensively with a subset of the group. Hence, overall display distinctiveness must be assessed using multiple variables simultaneously.

#### *DFA and individual distinctiveness*

To examine the individual distinctiveness of push-ups we employed DFA, a multivariate method used to assess the individual distinctiveness of visual displays in green iguanas (Dugan 1982), and of vocal signals in avian, mammalian and anuran species (e.g., Gelfand and McCracken 1986; Bee et al. 2001; Bard et al. 2002). The percentage of displays assigned to the correct individuals provides a measure of individual distinctiveness. DFA can assign displays to individuals using two predictive approaches. The training set approach employs one group of displays, known as a training set, in the construction of discriminant functions and then uses those functions to assign displays in a separate test set of observations. We used this approach to test the success of discriminant functions generated in one state in classifying push-ups produced in the alternate state. For each display the cross-validation approach generates discriminant functions using all of the displays except that display, and then assigns that display to an individual. We used this approach in analyses within states, or when both states were pooled.

The rate of classification to correct individuals, our measure of individual distinctiveness, is expected to decrease as the number of individuals included in a DFA increases (Beecher 1989). By including the maximum available number of individuals in our DFA we obtained a conservative estimate of individual distinctiveness. We employed a randomization approach to evaluate whether the percent of correct classification obtained in the DFA differed from that expected by chance. We used our data to generate 100 sets of data in which push-ups were randomly allocated to individuals. Each randomized data set was analyzed in a DFA, yielding null values of classification success.

### *Effects of sample size on classification success*

Discrimination should become more difficult as the number of individuals that must be recognized increases, but discrimination is improved as the number of displays per individual increases (Beecher 1989). To evaluate the potential of these variables to affect the rate of successful classification, we repeatedly selected random sub-samples of 3, 6 and 9 individuals from our original sample of 12 individuals. For each of the selected individuals we randomly selected a sub-sample of 5 push-ups from the original sample of 11 push-ups. We ran a DFA for each of 20 randomized data sets for each of the three sample sizes, using the cross-validation method of group assignment. The results of these runs were collated as means with 95% confidence intervals for each sample size. Confidence intervals for each mean rate of correct classification were constructed using 1,000 bootstrapped samples. These analyses were then repeated with all 11 push-ups per individual.

#### *Effect of fatigue on repeatability and classification success*

We assessed the resistance of display structure to the effect of fatigue using a sample of eight individuals that performed multiple push-ups in both rested and fatigued states. Our sample included 100 push-ups from the rested state and 47 push-ups from the fatigued state, which accounted for 68% and 32% of the sample, respectively.

To test the effect of fatigue on the stability of each display parameter, we computed repeatability values within each state (rested, fatigued) and repeatability values obtained from pooling push-ups from both states. If push-ups structure changes as a result of fatigue, then repeatability values calculated within state should be higher than repeatability values calculated with both states pooled. To calculate the probability with which repeatability values higher within days than with both days pooled could be expected by chance, we randomly shuffled the 24 obtained repeatability values 1,000 times (24 values obtained from eight push-up parameters, each calculated three times). In each reshuffled trial we counted the number of times that within state repeatabilities were higher than pooled repeatabilities, and divided by 1,000 the number of reshuffled trials in which these counts were equal to or greater than the number observed in our repeatability analysis.

We tested whether push-ups preserve their individually distinctive differences in spite of experimentally induced fatigue by comparing DFA classification success within state to classification success across states. Classification success across states was calculated using the push-ups from one state as the training set (which generates the discriminant functions) and push-ups from the alternative state as the test set (with which classification success is evaluated). These values were compared to classification success within the state that served as the training set and to two null models. First, we predicted that if individual distinctiveness is maintained between states, then randomly re-assigning push-ups to state should yield rates of correct classification similar to the rates observed in the original analysis. We generated 100 randomized data sets, by randomly re-assigning the state of each push-up in the original set of data, with probabilities that reflected the composition of the original data set. Second, the rates of correct classification expected if push-up structure was not individually distinctive were obtained from 100 runs of DFA in which the push-ups in our original data set were randomly reassigned to individuals.

#### *Can push-up structure reveal fatigue?*

For each push-up parameter we calculated mean values within state for each of the eight individuals that performed multiple push-ups in both states. For each parameter, the means were used in paired samples and in random samples *t*-tests to assess the influence of fatigue. We used DFA to evaluate how well push-ups could be assigned to the correct state when individual identity is disregarded.

The rates of correct classification obtained in this DFA were compared to the rates returned in 100 DFA runs in which push-ups were randomly re-assigned to state.

## Results

### Repeatability of display parameters

Repeatable variation among individuals was evident in all push-up parameters (Table 1). Repeatability values ranged from low to moderately high (0.08–0.68) and one-way ANOVAs showed that all the parameters differed significantly between individuals. *P*-values are all significant at the table-wide alpha of 0.05 after adjusting with the sequential Bonferroni procedure to take into account multiple tests (Rice 1989). The three variables that showed the highest repeatabilities were, in decreasing order: peak A duration, ascent duration and the relative height of peak B.

### Individual distinctiveness

Our DFA assigned 58.3% of push-ups to the correct individual, a rate that is much higher than the classification success rates obtained in 100 runs of DFA in which

push-ups were randomly re-allocated to individuals (median 8.3%, range 2.3% to 16.7%). The first three canonical functions accounted for over 82% of the variation (Table 2). Display parameters peak A duration, relative height of peak B and ascent duration had the highest correlation coefficients, respectively, with the first three canonical functions (Table 2).

### Effects of sample size on classification success

Repeated sub-sampling of individuals for DFA revealed a dependency of classification success on the number of individuals sampled when the analyses included 11 push-ups for each individual (Fig. 3). Median values for classification success decreased as the sample size changed from three to six to nine individuals (82.1%, 75.1%, 66.1%, respectively). In contrast, when we used 5 push-ups per individual there was no appreciable change across sample sizes of three, six and nine individuals (58.6%, 60.8%, and 59.1%, respectively). These results also show that classification success varies with the number of push-ups included in the DFA: for three, six and nine individuals, classification success was higher for analyses that included 11 push-ups per individual than classification success in analyses that included only five

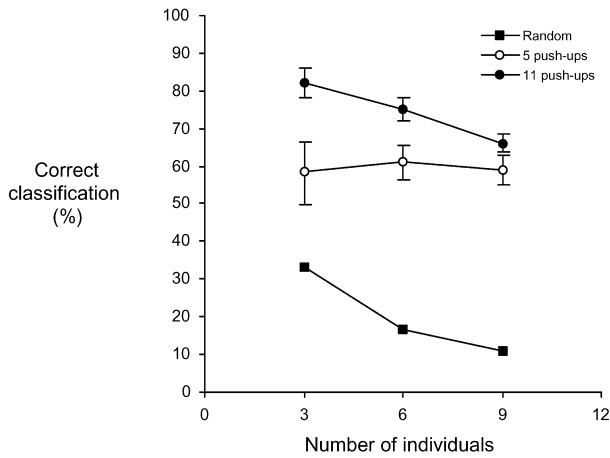
**Table 1** Descriptive statistics, repeatability analysis and ANOVA results of the push-up parameters for a sample of 132 push-ups (12 lizards, each with 11 push-ups)

Variable	Mean	Standard deviation	Minimum	Maximum	Repeatability	<i>F</i> statistic	<i>P</i> -value
Duration							
Pause	2.098	0.864	0.00	5.00	0.18	3.497	<0.001
Ascent	6.076	2.495	3.00	16.00	0.53	13.573	<0.001
Peak A	4.159	1.375	2.00	9.00	0.68	24.555	<0.001
Relative height							
Trough A	0.290	0.313	-0.63	0.84	0.22	4.040	<0.001
Peak B	0.446	0.314	-0.27	0.98	0.48	11.173	<0.001
Trough B	-0.004	0.225	-0.66	0.84	0.14	2.794	0.003
Peak C	0.135	0.250	-0.46	0.84	0.31	5.88	<0.001
Trough C	0.016	0.232	-0.93	0.84	0.08	1.990	0.035

**Table 2** Factor structure matrix for push-up parameters in a discriminant function analysis assessing individual distinctiveness (132 push-ups: 12 individuals, each with 11 push-ups). Values represent the pooled within-groups correlations between push-up parameters and standardized canonical discriminant functions. Eigenvalues, variance contributions, and canonical correlation coefficients are listed for each canonical discriminant function

	Canonical discriminant function							
	1	2	3	4	5	6	7	8
Push-up parameter								
Peak A duration	0.738*	0.375	-0.074	-0.397	0.142	-0.081	0.326	-0.136
Peak B	0.198	-0.547*	0.057	0.019	0.512	0.329	0.267	0.465
Ascent duration	0.33	0.169	0.817*	0.311	-0.2	-0.034	0.133	0.2
Trough B	0.03	0.113	-0.096	0.187	0.666*	0.056	0.584	0.393
Pause duration	0.141	0.154	-0.02	0.193	0.353	0.661*	-0.555	-0.221
Trough C	-0.002	0.086	-0.056	0.108	0.45	0.398	0.776*	0.121
Peak C	0.243	-0.147	-0.241	0.457	0.291	0.23	0.513*	0.501
Trough A	0.158	-0.295	0.004	-0.083	0.217	0.42	0.155	0.796*
Eigenvalue	3.192	2.58	1.093	0.812	0.352	0.226	0.072	0.004
% of Variance	38.3	31	13.1	9.7	4.2	2.7	0.9	0
Cumulative %	38.3	69.3	82.4	92.1	96.4	99.1	100	100
Canonical correlation	0.873	0.849	0.723	0.67	0.51	0.43	0.259	0.063

\* Largest absolute correlation between each variable and any discriminant function



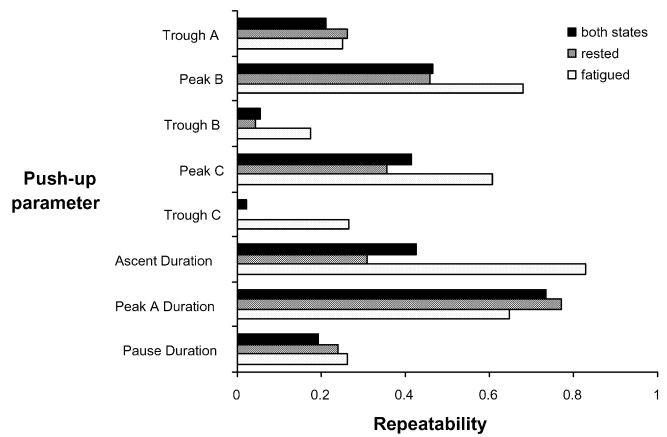
**Fig. 3** The effects sample size on classification success in DFA, showing the percentage of push-ups assigned to the correct individual. Individuals were repeatedly sub-sampled from a larger pool of 12 individuals. Median values and 95% confidence intervals are shown for the rates of correct classification obtained in analyses that included three, six or nine individuals and five or 11 push-ups per individual. The rate of correct classification expected by chance (100/N) is presented for comparison

push-ups per individual. Regardless of the number of individuals or of the number of push-ups included in the analyses, classification success was higher than expected by chance.

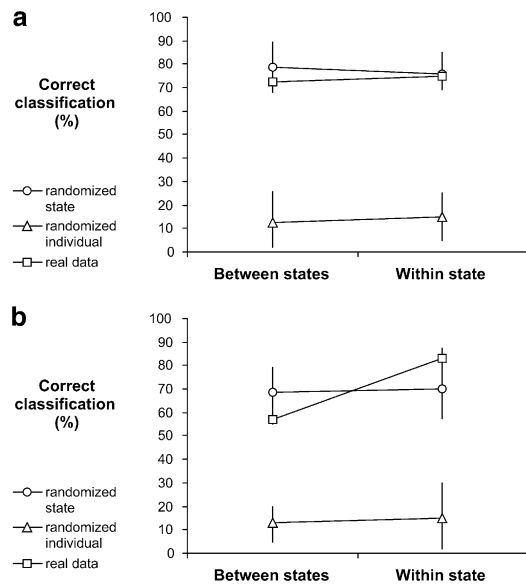
**Effect of fatigue on repeatability and classification success**

Repeatability analyses of parameters calculated separately for rested push-ups and fatigued push-ups yielded values higher than the repeatabilities of corresponding push-up parameters calculated with both states pooled, in 10 of 16 cases (Fig. 4). However, since 244 of 1,000 trials in which repeatability values were randomly reshuffled yielded frequencies of 10 cases or higher, repeatability values were not judged to be consistently higher when calculated within states than when calculated for both states pooled (estimated  $P=0.244$ ). Similarly, while we found that repeatability values calculated for fatigued push-ups were higher than the corresponding values calculated for rested push-ups in six of eight cases, this frequency was not statistically different than could be expected by chance ( $P=0.139$ ).

Using DFA, the values obtained in our comparison of classification success within and between states compared favorably with the values returned when push-ups were randomly re-assigned to state (Fig. 5). Both of these values were much higher than the values obtained in DFA runs in which push-ups were randomly re-assigned to individuals (Fig. 5). When push-ups performed in the rested state provided the training set and push-ups performed in the fatigued state served as the test set, the rate of assignment of fatigued push-ups to the correct



**Fig. 4** Repeatability values of push-up parameters for eight individuals that performed multiple push-ups in both rested and fatigued states, comparing repeatability within each state to repeatability with push-ups from both states pooled



**Fig. 5a, b** Percentage of push-ups assigned to the correct individual in discriminant function analyses within and between states (rested or fatigued). The real values (black boxes) are compared with the median and range of values obtained from 100 analyses in which push-ups were randomly re-assigned to state (grey circles) and from 100 analyses in which push-ups were randomly re-assigned to individual (grey triangles). In each analysis the push-ups within one state provided the training set for generating the discriminant functions. **a** Rested push-ups provided the training set, with fatigued push-ups providing the test set. **b** Fatigued push-ups provided the training set, with rested push-ups providing the test set

individual was similar to the rate of correct assignment within rested push-ups (72.3% and 75.0%, respectively, Fig. 5a). When fatigued push-ups served as the training set and rested push-ups provided the test set, the rate of assignment of push-ups performed in the rested state to the correct individual was somewhat lower than the rate of correct assignment of fatigued push-ups (57.0% and

**Table 3** independent and paired-samples *t*-tests examining the effect of state (rested vs fatigued) on push-up parameters of 8 individuals, in which each parameter was averaged within each individual and state

Push-up parameter	Independent-samples <i>t</i> -test		Paired-samples <i>t</i> -test	
	<i>t</i>	<i>P</i> -value	<i>t</i>	<i>P</i> -value
Duration				
Pause	-0.154	0.880	0.184	0.859
Ascent	0.346	0.734	-0.541	0.605
Peak A	0.244	0.811	-0.817	0.441
Relative height				
Trough A	-1.567	0.139	2.217	0.062
Peak B	-1.379	0.190	4.248	0.004*
Trough B	-1.316	0.209	1.434	0.195
Peak C	-0.833	0.419	1.851	0.107
Trough C	-0.959	0.354	0.992	0.354

\* *P*-values that remain above a tablewide significance level of 0.05 after employing the sequential Bonferroni adjustment

83.3%, respectively; Fig. 5b). Nonetheless, both values were encompassed within the range of values returned by DFA runs in which push-ups were randomly reassigned to state.

#### Can push-up structure reveal fatigue?

When analyzed as independent-samples *t*-tests, state did not significantly affect any push-up parameter (Table 3). In contrast, when analyzed as a paired-samples *t*-test, in which samples were paired by individual, the relative height of peak B was significantly higher in fatigued push-ups, while all other push-up parameters did not differ significantly between states (Table 3). After sequential Bonferroni correction for multiple tests (Rice 1989), the relative height of peak B remained significantly different between states. Using DFA to assign push-ups to state yielded a success rate of 63.9%, well within the rates returned in DFA runs in which push-ups were randomly reassigned to state (median 66.0%, range 61.2–70.1%).

## Discussion

To our knowledge, the current study is among the first to directly examine the effect of fatigue on any signal characteristics, and the first to directly test the resistance of an individually distinctive signal to fatigue. Previous studies have attributed changes in signaling rate and intensity during the course of a signaling episode to fatigue, but did not manipulate fatigue directly (Briffa et al. 2003). Among lizards, both the duration of the laterally compressed threat posture and the rate of push-up production decreased in response fatigue (Brandt 2003). In contrast, diverse analyses conducted in the present study consistently showed that in male side-blotched lizards, push-up structure is individually distinctive and

resistant to the impact of fatigue. Our results are therefore consistent with the hypothesis that push-up can mediate social recognition in lizards. Individual recognition may underlie ‘dear enemy’ relationships among territorial lizards, dominance hierarchies, and mate recognition, allowing lizards to target appropriate behavioral responses to specific social partners. In the future, this hypothesis can be directly tested in video playback experiments.

We tested whether push-ups maintain their individually distinctive signatures using DFA, in which displays from one state (e.g., rested) generated discriminant functions for assigning displays produced in the alternate state (e.g., fatigued). Because classification success between states was comparable to classification success within each state, we concluded that push-up distinctiveness is resistant to fatigue. Similarly, repeatability values did not vary consistently between states, or when states were pooled. The resistance of push-up structure to fatigue-induced changes was surprising, considering that fatigue is known to affect the contractile properties of skeletal muscle (MacIntosh and Allen 2000). However, motor control systems are generally capable of compensating for the reduced responsiveness of fatigued muscle by increasing the levels of stimulation, thus minimizing the variation in movement characteristics (e.g., Corcos et al. 2002).

We also directly investigated the potential of push-ups to reveal fatigue. None of the push-up parameters measured in the current study varied significantly between the rested state and the fatigued state when analyzed as independent-samples *t*-tests. In contrast, the same data analyzed in paired-samples *t*-tests revealed a significant difference between states for one of the push-up parameters: the relative height of peak B. This comparison revealed that push-up state could be distinguished only when the individual that produced the push-up was taken into account. A DFA, which pooled all individuals classified push-ups to the correct state at rates that did not differ from rates returned by analyses of data sets in which push-ups were randomly re-assigned to state. We therefore conclude, for the parameters examined in the present study, that push-up structure can not reveal fatigue independently of individual identity.

To determine whether push-up structure is individually distinctive, or alternatively whether variation is encompassed by a few discrete push-up morphs (McKinney 1971a, 1971b), we conducted an analysis that used the maximal available number of individuals, disregarding their state of fatigue. The classification success rate in our sample of 12 individuals were much higher than expected by chance, suggesting that push-ups are individually distinctive. The potential of push-ups to encode individual identity is dependent on the sensitivity of our estimate of distinctiveness to variation in the number of push-ups and the number of individuals included in our analyses. Rates of correct classification in DFA are generally expected to increase as the number of individuals included decreases, and as the number of displays per individual increases (Beecher 1989; Bee et al. 2001). Repeated sub-sampling

of our data revealed that when a sufficient number of push-ups were included in the analysis, classification success rates do increase as the number of individuals included in the analysis decreases. When fewer push-ups are included in the analysis, successful classification was lower overall, and failed to respond to variation in the number of individuals included in the analysis.

Our results suggest that push-ups, while individually distinctive, may not suffice for reliably recognizing individuals when performed singly and in the absence of other cues, particularly if a large number of individual must be discriminated. However, during social interactions, lizards employ multiple push-ups: in the field, introduction of a tethered male intruder resulted in residents performing an average of 21 (range 4–41) push-ups prior to attack (Brandt and Sinervo, unpublished data). Lizards might achieve higher rates of correct classification than in our DFA by using a more comprehensive array of push-up characteristics, evaluating the existing push-up parameters more precisely than we did, or employing additional sources of information independent of push-up structure. Individual variation in color patterns and chemical signatures may also contribute towards individual recognition in lizards.

Several factors that may reduce the individual distinctiveness of the push-up display were not addressed by the present study. First, if push-up structure varies with temperature then the potential of push-up distinctiveness to promote individual discrimination may be smaller than our study indicates. Hypothermic conditions have been shown to prolong display sub-units in the ‘signature bob’ of *Iguana iguana*, although amplitude was not affected by hypothermia (Phillips 1995). However, many diurnal heliothermic lizards regulate field activity temperatures to a narrow range (Huey 1982). Furthermore, within several degrees of the preferred temperature there is often little effect of temperature variation on locomotor performance (Bennett 1980). We therefore suggest that under field conditions there may be little scope for temperature variation to affect push-up structure to a degree that would compromise the individual distinctiveness of push-ups. An additional factor that was excluded from the current study is the potential effect of variation in context and motivation on push-up structure (Martins 1993). Our experiment was designed to minimize the variation in these variables, in order to reduce the possibility that differences in context and motivation would falsely generate individual differences. The effect of contextual variation on the individual distinctiveness of push-up structure could be addressed directly by recording push-ups from known individuals in multiple contexts.

In summary, the present study showed that several duration and relative amplitude parameters of the push-up display exhibit repeatable variation among individuals. In combination, these differences comprise individually distinctive display patterns that can be used to reliably assign push-ups to the individual that produced the push-ups. For the variables examined in the present study, push-up structure was found to be robust to the effects of

experimentally imposed fatigue. These results are consistent with the hypothesis that subtle individually distinctive characteristics of push-up structure play a role in social recognition in lizards.

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