



Mechanisms of decision making during contests in green anole lizards: prior experience and assessment



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Prior contest experiences can predictably alter an individual's contest performance and probability of contest success. Although winner and loser effects have been well studied across many animal taxa, the mechanisms underlying these effects and their adaptive value currently are topics of intense interest. Two predominant hypotheses posit that contest experiences alter either an individual's perceived fighting ability or its actual fighting ability. We addressed these hypotheses, and potential physiological and behavioural mechanisms driving experience effects in the green anole lizard, *Anolis carolinensis*. Prior losers went on to lose a significant proportion of future contests while prior winners were equally likely to win or lose against size-matched opponents. Further analysis revealed that the loser effect arose as a result of individuals updating their perceived fighting ability following a loss. Both prior losing and winning experiences influenced future contest performance with prior losers decreasing and prior winners increasing their aggressiveness in subsequent contests. Status-dependent changes in metabolic physiology were not associated with the presence of the observed loser effect. However, contest status and contest performance interacted to influence metabolic physiology. Plasma glucose concentrations decreased as a function of the frequency of high-risk, escalated behaviours performed by eventual losers, and muscle lactate concentrations increased as a function of the frequency of low-risk threat displays performed by eventual winners. Our results support the notion that prior contest experiences influence an individual's perceived, not actual, fighting ability and that status-dependent changes in metabolic physiology are not a likely mechanism underlying the presence/magnitude of experience effects.

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Prior contest experiences predictably alter an animal's decisions during competitive interactions; prior wins increase while prior losses decrease an individual's aggressiveness in subsequent contests (reviewed in: Hsu, Earley, & Wolf, 2006; Rutte, Taborsky, & Brinkhof, 2006). Such behavioural changes can manifest as winner and loser effects, defined as any change in contest performance and probability of contest success following a win or loss (Chase, Bartolomeo, & Dugatkin, 1994; Goubault & Decuigniere, 2012; Hsu et al., 2006; Huang, Yang, & Hsu, 2010; Kasumovic, Elias, Sivalinghem, Mason, & Andrade, 2010; Schuett, 1997). Experience effects often are short-lived, persisting from hours (e.g. pumpkinseed sunfish, *Lepomis gibbosus*: Chase et al., 1994) to days (e.g. copperhead snakes, *Agkistrodon contortrix*: Schuett, 1997), and sometimes up to 1 month (Lan & Hsu, 2011). Furthermore, the effects of prior contest dynamics (e.g. escalated versus nonescalated) on future contest behaviour and success often can supersede the

effects of prior status alone (Beaugrand & Goulet, 2000; Garcia et al., 2012; Huang et al., 2010).

Despite the breadth of information on winner and loser effects, the proximate and ultimate mechanisms underlying these experience-induced behavioural changes have yet to be elucidated. Some theorize that winner and loser effects play a pivotal part in the formation of social hierarchies (Dugatkin, 1997; Dugatkin & Earley, 2003; Hock & Huber, 2009). Hock and Huber (2009) demonstrated that the presence of strong loser effects and weak winner effects could reduce the frequency of costly aggressive interactions between group members and lead to the formation of stable hierarchies. Others theorize that prior contest experiences provide animals with information regarding their resource-holding potential; the culmination of factors (e.g. size, motivation, residency, etc.) that aid an individual in obtaining or retaining fitness-related resources (Fawcett & Johnstone, 2010; Hsu et al., 2006; Mesterton-Gibbons, 1999; VanDoorn, Hengeveld, & Weissing, 2003a, 2003b). These models predict that selection should favour individuals that utilize prior contest experiences to reassess their fighting ability under conditions where asymmetries in resource-holding potential dictate contest outcome, where information of

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one's own resource-holding potential may be imperfect (VanDoorn et al., 2003a, 2003b), and when overestimating resource-holding potential carries significant costs (Mesterton-Gibbons, 1999). Prior contest experiences should thus influence an animal's perceived fighting ability but not its actual fighting ability (Hsu et al., 2006; Hsu, Lee, & Lu, 2009; Hsu & Wolf, 2001; but see Kasumovic et al., 2010). These modifications should affect contest performance and success only in nonescalated contests, during which an animal's threshold fighting ability is not revealed and does not influence contest outcome (Hsu et al., 2006, 2009).

A change in perceived fighting ability has often been cited as the mechanism underlying winner and loser effects, but alternative mechanisms have been proposed (Hsu, Earley, & Wolf, 2011; Rutte et al., 2006). Among those alternatives is the by-product hypothesis, which states that an animal's actual fighting ability can change following a win or loss. Animals that win gain fitness-related resources (e.g. food), which increase their resource-holding potential while losers incur energetic costs or injury, which decrease their resource-holding potential. Winner and loser effects would then arise as a by-product of those changes in actual fighting ability (Hsu et al., 2011; Rutte et al., 2006). Rutte et al. (2006) indicated that the adaptive value of by-product experience effects remains unclear, especially for the loser, and noted that winner and loser effects have been revealed in the absence of resource gain or loss (e.g. Bergman et al., 2003; Chase et al., 1994; Schuett, 1997). However, in jumping spiders, *Phidippus clarus*, prior contest experience affects performance in both nonescalated and escalated contests, perhaps by altering both perceived and actual fighting ability (Kasumovic et al., 2010). This study, however, did not address the mechanism(s) underlying potential changes in actual fighting ability and, to the best of our knowledge, no study has investigated the potential link between changes in actual fighting ability following a contest and its effects on future contest performance and success.

Our study explored whether changes in metabolic physiology (by-product hypothesis) or perceived fighting ability (perceived ability hypothesis) following a prior win or loss are associated with changes in an animal's performance in subsequent contests. Although we draw a distinction between the two hypotheses, they need not be mutually exclusive (Kasumovic et al., 2010). We chose metabolic physiology because it has been shown to be a significant component of an individual's resource-holding potential in many taxonomic groups (reviewed in Briffa & Sneddon, 2007; see also Brandt, 2003; Copeland, Levay, Sivaraman, Beebe-Fugloni, & Earley, 2010; Milligan, 1996; Ros, Becker, & Oliveira, 2006). The rate at which energy is spent and lactic acid is accumulated influences whether an individual persists in or retreats from a contest (Briffa & Elwood, 2001; Briffa & Sneddon, 2007; Wilson & Gatten, 1989). Changes in metabolic physiology commonly are observed after agonistic contests in both winners and losers (Briffa & Sneddon, 2007; Copeland et al., 2010), but these status-dependent changes have yet to be linked to changes in future contest success.

We hypothesized that the outcome and dynamics of prior contests, but not status-dependent changes in metabolic physiology, would predict the probability of future contest success. We predicted that experience effects (winner and/or loser) would persist longer than any status-related changes in metabolic physiology. Although we hypothesized that status-dependent changes in metabolic physiology would not affect future contest success, we expected that acute changes in metabolic physiology would occur in response to an initial contest (e.g. Wilson & Gatten, 1989). We also expected that any observed metabolic changes would be a function of both prior contest status (winner versus loser) and prior contest dynamics (escalated versus nonescalated). As such, we hypothesized that focal individuals would show significant changes in metabolic physiology (e.g. decreased plasma glucose or

increased muscle lactate) following the initial contest and that the magnitude of these changes would depend upon both prior contest status and dynamics.

We used the green anole lizard, *Anolis carolinensis*, as a model organism. For male green anoles, reproductive opportunities depend critically on their ability to successfully establish and defend a high-quality territory (Forster, Watt, Korzan, Renner, & Summers, 2005; Jenssen, Decourcy, & Congdon, 2005; Korzan, Øverli, & Summers, 2006; Lovern & Jenssen, 2003). In the wild, neighbouring males often compete in dyadic interactions at their respective territory boundaries (Jenssen, Greenberg, & Hovde, 1995). Competitive interactions follow a phasic pattern (Jenssen et al., 2005) starting with highly ritualized display tactics (e.g. headbobs, dewlap extensions, colour changes, eyespot formation), which may escalate to high-risk behaviours (e.g. charging, mouth locking, wrestling) until one of the contestants retreats (Henningsson & Irschick, 2012; Lailvaux, Herrel, VanHooydonck, Meyers, & Irschick, 2004; Lailvaux & Irschick, 2007; McMann, 1993). Interactions between green anoles can elicit significant changes in metabolic physiology (e.g. lactate accumulation and oxygen consumption; Wilson & Gatten, 1989; but see Wilson, Gatten, & Greenberg, 1990) and neuroendocrine profiles (e.g. serotonin and cortisol; Korzan et al., 2006; Ling, Summers, Renner, & Watt, 2010). Success in dominance interactions also is linked to individual performance capacity (e.g. bite force or jumping ability: Henningsson & Irschick, 2012; Lailvaux et al., 2004; Lailvaux & Irschick, 2007), and several studies have revealed that the decisions green anoles make during contests depends on prior contest experiences. Forster et al. (2005) showed that individuals confronted with an opponent to whom they had previously lost perform fewer aggressive acts and retreat faster (see also Larson & Summers, 2001; Ling et al., 2010). Garcia et al. (2012) showed that the interaction between prior contest performance and status can significantly influence an individual's competitive success when faced with a novel opponent.

Our choice of model organism and experimental set-up has given us the added advantage of re-evaluating previous findings on assessment strategies in green anole lizards. Previous work suggests that green anoles use different assessment strategies, either self-assessment or mutual assessment, depending on the intensity of a contest (Garcia et al., 2012; but see Henningsson & Irschick, 2012). In low-intensity, nonescalated contests, individuals use a mutual assessment strategy, but in high-intensity, escalated contests, individuals use a self-assessment strategy (Garcia et al., 2012). When using mutual assessment, individuals gather information and gauge asymmetries in resource-holding potential through behavioural exchanges; contests escalate to high-risk behaviours when asymmetries in resource-holding potential cannot be determined through low-risk displays alone (e.g. Enquist, Leimar, Ljungberg, Mallner, & Segerdahl, 1990). Conversely, when using self-assessment, individuals base their competitive decisions solely on assessment of their own resource-holding potential, often irrespective of their opponents' actions (pure self-assessment: Arnott & Elwood, 2008; Briffa, 2008; Briffa & Elwood, 2010; Mesterton-Gibbons, Marden, & Dugatkin, 1996; Payne & Pagel, 1996), but not always (i.e. cumulative assessment: Payne, 1998). We thus hypothesized that individuals would use different assessment strategies (e.g. mutual versus self-assessment) depending on the intensity of the contest in which they engaged.

METHODS

Animal Housing and Care

All procedures were approved by the University of Alabama Institutional Animal Care and Use Committee (IACUC number 08-

315-01). Only adult male green anoles were used. Shipments of 72 field-caught males arrived every 3 weeks from Charles D. Sullivan's Reptile Distributors (Nashville, TN, U.S.A.); field collection sites were located throughout southern Louisiana (information provided by distributor). All animals were housed in isolation for a minimum of 7 days before use. Individual housing was handmade and consisted of sealed wooden terrariums (12.5 × 12.5 × 12.5 cm) with window screen mesh backs and doors to allow ventilation and light to pass through. Terrariums were filled with a 3 cm deep layer of Repti Bark natural bedding and had fake leaves and branches for enrichment. Room temperature was maintained at 27 °C and lighting was provided by Reptisun 5.0 UVB bulbs (2400 lm) on a 14:10 h light:dark cycle. All animals were watered with a spray bottle and fed two medium-sized crickets covered in vitamin D powder twice daily. This study was performed during July–September in 2009 and 2010, which spans the latter half of the green anole breeding season (Jenssen et al., 1996; Jenssen & Nunez, 1998; Licht, 1971, 1973).

Measurements and Contest Set-up

After 7 days of acclimation/isolation, each animal was removed from its housing tank, weighed (g) and measured (snout–vent length, SVL, in mm) (Table 1). A subset of the largest (standard winner) and smallest (standard loser) animals were chosen to provide experiences to the focal animals; standard winners provided losing experiences and standard losers provided winning experiences to the focal individuals using a random-selection procedure (Hsu et al., 2006). A total of 12 standard winners and 12 standard losers were used and were given 1–2 weeks between uses for recovery. All other animals were assigned a number and were size-matched with a naïve opponent that differed by no more than 0.1 g body mass. Animals were randomly assigned as subjects that would receive a winning experience against a standard loser (designated winners), a losing experience against a standard winner (designated losers), or no experience (control). One day before the primary contest, two designated winners, two designated losers and two controls were moved from isolation into separate fight tanks. Fight tanks were handmade sealed wooden terrariums (62 × 31 × 31 cm), divided into three equally sized compartments using wooden dividers, with a window screen mesh top and a clear Plexiglas front. All focal animals (designated winner, designated loser, control) were placed on the left side of the fight tank. We placed a standard loser (at least 1 g smaller than the designated winner), a standard winner (at least 1 g larger than the designated loser), or no opponent (control) into the right compartment of the fight tank. The central compartment was left unoccupied. All animals were fed and watered (see *Animal Housing and Care* above) during a 1-day acclimation period.

Behavioural Trials

After the 1-day acclimation period, we began primary contests each day at 1100 hours by removing the partitions separating the

contestants. Contests continued until one male rapidly retreated (settlement). We recorded all contests on digital video from behind a blind. Control individuals were kept in an open fight tank with partitions removed, but no opponent present, for the same amount of time as a designated fight that was run simultaneously. Once contests had reached settlement, individuals were allowed to freely interact for a 10 min postsettlement period, after which time they were returned to isolation. The postsettlement period was used to ensure that there were no reversals in contest outcomes; no outcome reversals were observed. Fight tanks were cleaned, substrate was removed and individuals were placed in unfamiliar tanks to eliminate residual cues that could bias future behaviour in the secondary contests. Designated individuals were either returned to the left-hand side of a fight tank or processed for tissue samples (see *Tissue Collection*) depending on the treatment assigned (see *Treatments*). Size-matched, naïve opponents (≤ 0.1 g asymmetry) were placed in the opposite side of the fight tank in preparation for the secondary contest. Both focal and naïve individuals spent 2 days in the fight tank prior to the secondary contest and were fed and watered as described above. Secondary contests were held 2 days following the primary contest at 1100 hours. Each secondary contest was digitally recorded and conducted in exactly the same manner as the primary contest except for controls, which now faced a size-matched opponent. After the 10 min postsettlement period, all individuals were transferred to a processing station where tissue samples were collected (see *Tissue Collection*). Digital recordings of all contests were scored for behaviours summarized in *Supplementary Table S1* using the JWatcher program (<http://www.jwatcher.ucla.edu/>). J.M., who was blind to the hypotheses tested, scored all primary and secondary contests.

Treatments

We randomly assigned designated winners, designated losers and controls to one of three treatments prior to the primary contest. In treatment 1, focal individuals were processed for tissue samples (see *Tissue Collection*) immediately following the primary contest without being returned to the fight tanks or given a secondary fight. This treatment was used to quantify metabolic physiology immediately following the primary contest. In treatment 2, focal individuals were returned to the right-hand compartment of the fight tanks following the primary contest and remained in the fight tank for 2 days. Following the 2-day waiting period, individuals were removed and processed for tissue samples without being given a secondary contest. This treatment was used to quantify decay of any changes in metabolic physiology that occurred in response to the primary contest. In treatment 3, designated individuals competed in both primary and secondary contests before being processed for tissue samples. The final treatment was used to quantify the effects of fighting experience on future contest performance and outcome as well as the effects of two consecutive contests on metabolic physiology. Our choice of a 2-day time interval was based conservatively on previous work in copperhead snakes, which showed that experience effects can persist up to 7 days in reptiles (Schuett, 1997), and in green anoles, which showed that the animals can recognize prior opponents up to 7 days (Forster et al., 2005). In a prior study (Garcia et al., 2012), we found no experience effects in the green anole at a 2-day time interval. However, in the present study, we used a protocol that was optimized (e.g. increased arena size) based upon what we learned from our previous work. The sample sizes for each treatment were as follows: treatment 1 (14 designated losers, 17 designated winners, 14 controls; 45 total); treatment 2 (17 designated losers, 17 designated winners, 19 controls; 43 total);

Table 1
Mean mass and standard length for all designated individuals, control individuals, standard individuals, and naïve opponents

Designation	Mean±SE (range)	
	Mass (g)	SVL (mm)
Designated winner	4.71±0.69 (2.98–6.26)	57.99±3.32 (43.5–64.4)
Designated loser	3.97±0.55 (2.88–5.25)	54.83±3.65 (43–63.8)
Control	4.44±0.68 (2.82–6.06)	56.65±3.32 (44.4–64.1)
Standard winner	5.37±0.57 (4.31–6.87)	60.24±3.11 (51.5–67.5)
Standard loser	3.47±0.63 (2.2–5.83)	53.25±2.99 (44.5–63.3)
Naïve	4.27±0.72 (2.71–5.97)	56.25±2.86 (51.3–63.3)

treatment 3 (21 designated winners, 21 designated losers, 16 controls; 58 total).

Tissue Collection

Animals were euthanized prior to tissue extraction with rapid decapitation and pithing. This procedure has been approved by the [American Veterinary Medical Association \(2007\)](#) euthanasia guidelines for reptiles. We collected trunk blood samples using heparinized capillary tubes, then expelled each sample into a 0.5 ml microcentrifuge tube and stored it on ice. Blood was centrifuged in an Eppendorf 5418 centrifuge at 5400 g for 2 min to obtain plasma, which was transferred to new 0.5 ml microcentrifuge tubes and stored at -80°C . We opened the abdominal cavity and excised the liver tissue, then placed the sample in a 1 ml centrifuge tube, fast-froze it in liquid nitrogen and stored it at -80°C . We cleared the abdominal and thoracic cavities of all other organs before placing the entire body in a 50 ml Falcon tube, then submerged it in liquid nitrogen and stored it at -80°C .

Plasma Glucose Assay

We assayed plasma glucose using reagents supplied with the Sigma-Aldrich Plasma Glucose (HK) Assay Kits (catalogue no.: GAHK-20). We removed the plasma samples from -80°C , thawed them at 4°C and centrifuged them at 5400 g for 2 min. We distributed 10 μl of standards (provided with the kit) or undiluted plasma samples across six 96-well Microtiter UV plates (Thermo Fisher Scientific Inc., Pittsburgh, PA, U.S.A.) followed by 200 μl of Assay Reagent; this hexokinase assay results in the production of NADH, which can be analysed spectrophotometrically. We incubated the plates on an orbital shaker at room temperature for 15 min and determined glucose concentrations (mg/ml) following reads at 340 nm using a Biotek ELx808 microplate reader. We converted glucose concentrations to mg/dl for analysis.

Lactate Assay

We assayed hepatic and muscle L-lactate with reagents supplied by Trinity Biotech (Lactate Reagent, catalogue no.: 735-10; Lactate Standard Solution, catalogue no.: 735-11). L-lactate was assayed according to [Bergmeyer \(1983\)](#) with modification. We excised 100 mg of muscle tissue from the pectoral region near the upper limbs and transferred the muscle to a 1 ml flat-bottom centrifuge tube. Anole 'headbob' and 'pushup' displays are produced using muscles of the pectoral region and are likely to show the greatest signs of metabolic change ([Decourcy & Jenssen, 1994](#)). We weighed liver tissue (mg) and transferred it to 1 ml flat-bottom centrifuge tubes. We homogenized the tissue samples (muscle or liver) in 1 ml of ice-cold 8% perchloric acid (with 1 mM EDTA) solution using a motor homogenizer (Fisher Scientific PowerGen Model 125, catalogue no.: 14-261-02), vortexed the samples for 10 s, incubated them on ice for 45 min and centrifuged them at 9000 g at 4°C for 5 min. We extracted a 200 μl aliquot, neutralized it using a KOH solution and recentrifuged it at 9000 g. We transferred a 50 μl aliquot into a new centrifuge tube and stored it at -80°C until assays were performed. To assay L-lactate, we added 250 μl of hydrazine/glycine buffer (promotes unidirectional conversion of lactate to pyruvate) to all test wells on 96-well UV plates, then added 20 μl of diphosphopyridine nucleotide (5×10^{-2} β -DPN) solution, as a source of NAD^{+} , and 20 μl of standards or samples. We incubated the plates for 15 min on an orbital shaker at room temperature. We took background spectrophotometric readings (340 nm) three times at 3 min intervals. Next, we added 2 μl of lactate dehydrogenase to each well and incubated the plates for an

additional 20 min on an orbital shaker. We took a final reading, then subtracted the results of this reading from the initial background readings to determine lactate concentration (mg/dl) against a standard curve. Liver lactate concentrations were corrected for liver mass. No corrections were made for muscle lactate because muscle sample mass was standardized.

Statistical Analysis

We performed statistical analyses using SAS (version 9.1, SAS Institute Inc., Cary, NC, U.S.A) and JMP (version 7.0.1, SAS Institute Inc., Cary, NC, U.S.A). We used two-tailed heterogeneity *G* tests with Williams corrections to analyse the effects of (1) primary contest treatment (win, loss, control) on secondary contest outcome and (2) initiation and escalation (discrete variables; yes or no) in the primary and/or secondary contest on secondary contest outcome and performance. Linear regression determined the effects of absolute mass as well as mass asymmetries on contest duration in the primary contests. Mass and SVL were highly correlated with one another for designated losers and designated winners ($F_{1,106} = 161.96$, $P < 0.001$). Thus, we chose to report the results for mass because it has historically been the parameter used in the analysis of assessment strategies ([Taylor & Elwood, 2003](#)). Controls did not compete in a primary contest and were thus excluded from analyses of assessment strategy. Only primary contests were considered for analyses of assessment strategy because mass asymmetries existed; contestants were size-matched in the secondary contests. We determined the effects of treatment, status and treatment-status interaction on mean plasma glucose, muscle lactate and liver lactate using a MANOVA; liver and muscle lactate were ln transformed to achieve normality. To determine what drove significant treatment*status effects, we used planned comparisons (linear contrasts) with sequential Dunn–Šidák adjustments. We used principal component analyses (PCA) to distill 18 behavioural variables (e.g. approach, bite, wrestle, etc.; see [Supplementary Table S1](#)) to five principal components from the primary contest. Principal components retained for analysis had an eigenvalue greater than 1.0 (see [Table 2](#)). We compared plasma glucose, ln-transformed hepatic lactate and ln-transformed muscle lactate concentrations to behaviour scores using pairwise correlations.

RESULTS

Contest Outcome

Designated winners (individuals paired with a significantly smaller opponent) won a significant proportion of their primary contests (51 out of 64; $G_1 = 25.99$, $P < 0.001$) while designated losers (individuals paired with a significantly larger opponent) lost a significant proportion of their primary contests (48 out of 55; $G_1 = 34.01$, $P < 0.001$). These results validate our use of standardized opponents with a mass asymmetry of at least 1 g to provide focal individuals with a predetermined experience. Our subsequent analyses on experience effects were performed on (1) individuals that won (designated winners and designated losers that won) or lost (designated winners and designated losers that lost), or (2) individuals that obtained the intended experience (only designated losers that lost and designated winners that won). For ease of reading, we refer to individuals that won or lost, regardless of designation, as winners and losers, respectively. We refer to individuals that obtained their intended winning or losing experience as the designated winner and designated loser, respectively.

There was a significant difference between winners, losers and controls in their probability of future contest success ($G_2 = 9.10$,

Table 2
Summary of principal component analysis (PCA) on the behavioural measures of the primary contest

Behaviour	Settlement	Loading				
		PC1	PC2	PC3	PC4	PC5
Headbob	Pre	-0.01882	0.8523437	0.3288032	-0.111885	0.0043892
	Post	0.8393745	0.0714458	-0.028957	0.0129216	0.0000449
Lateral compression	Pre	0.2660956	0.5415108	0.2158584	-0.218729	-0.154617
	Post	0.820359	0.0272111	-0.08149	-0.083082	-0.079417
Dewlap	Pre	0.0344344	0.8482713	-0.096088	-0.121092	0.0936403
	Post	0.7926631	0.1606251	-0.033292	-0.126582	0.1581737
Approach	Pre	0.0630617	0.5664987	0.5414394	-0.105805	-0.15126
	Post	0.7238852	-0.072807	0.1184819	-0.191188	0.123715
Avoid	Pre	-0.223651	0.0140355	0.4152343	0.1794592	-0.193401
	Post	-0.058687	-0.10171	-0.051886	0.5969537	-0.034245
Lunge/charge	Pre	0.0260187	0.4249486	0.646793	-0.024902	0.2893067
	Post	0.2544136	0.1061894	-0.071178	-0.129516	0.7709122
Bite	Pre	0.0275407	0.0685236	0.0133903	-0.091494	0.1217716
	Post	-0.078407	-0.103195	0.0739938	-0.028021	0.8287031
Mouthlock	Pre	-0.045194	0.0817405	0.7328316	-0.074663	0.0072312
Wrestle	Pre	-0.019205	0.0602847	0.8006591	-0.07315	-0.038245
Retreat	Pre	-0.278763	-0.261552	-0.079619	0.7218266	-0.131183
	Post	-0.028121	-0.030689	-0.015775	0.7766575	-0.01558
Eigenvalue		4.002	3.088	1.511	1.411	1.058
% Variation		22.23%	17.16%	7.84%	6.32%	5.88%
Cumulative % variation		22.23%	39.39%	55.82%	61.94%	67.81%

Heaviest loadings on each component are shown in bold. Settlement represents whether behaviours were performed before or after contest settlement. Interpretations of each component are as follows: PC1: postsettlement, low-risk aggression; PC2: presettlement, low-risk aggression; PC3: presettlement, high-risk aggression; PC4: submission; PC5: postsettlement, high-risk aggression. % Variation: percentage of variation in the observed behavioural variables explained by each principal component (PC). Cumulative % variation: sum total percentage of variation in the observed behavioural variables explained by a given PC and its preceding PCs.

$P = 0.014$). Losers were significantly more likely to lose the secondary contest (19 out of 21 lost again; $G_1 = 15.53$, $P < 0.001$) while winners were equally likely to win or lose the secondary contest (11 out of 21 won again; $G_1 = 0.05$, $P = 0.83$). Controls had an equal probability of winning or losing the secondary contest (7 of 16 contests won; $G_1 = 0.24$, $P = 0.63$). To examine whether an individual's perceived and/or actual fighting ability was altered by their prior experience, we separated secondary contests into two categories (escalated or nonescalated) and examined the effects of prior contest experience on contest success in both categories (Hsu et al., 2006; Hsu & Wolf, 2001; Kasumovic et al., 2010). Contests were considered to have escalated when one or both contestants performed high-risk behaviours detailed in [Supplementary Table S1](#) (Henningsen & Irschick, 2012; Jenssen et al., 2005; Lailvaux et al., 2004; Lailvaux & Irschick, 2007; McMann, 1993). The results for nonescalated secondary contests were identical to the overall analysis: losers were significantly more likely to lose, while winners had an equal probability of winning or losing nonescalated secondary contests (losers: 15 of 17 contests lost; $G_1 = 11.3$, $P < 0.001$; winners: 4 of 10 contests won; $G_1 = 0.60$, $P = 0.44$). Control individuals had an equal probability of winning or losing nonescalated secondary contests (5 of 11 contests won; $G_1 = 0.67$, $P = 0.41$). Analyses of individuals that obtained their designated experience produced identical results, with designated losers losing 88% of the nonescalated secondary contests (14 of 16; $G_1 = 9.82$, $P < 0.002$) and designated winners winning 58% of the nonescalated secondary contests (10 of 17; $G_1 = 0.52$, $P = 0.47$).

Too few secondary contests escalated (9 for previous winners, 3 for previous losers and 2 for controls) to determine how prior contest experience affected outcome in escalated secondary contests.

Contest Performance

Individuals (pooled designated winners and designated losers) that initiated the primary contest were more likely to win than noninitiators ($G_1 = 28.70$, $P < 0.001$); initiators and noninitiators

represent individuals from independent contests and thus are mutually exclusive. This result was driven largely by the overwhelming propensity for designated winners to both initiate (41 of 60 contests initiated) and to win (40 of 41 initiators won); designated losers initiated 16 of 51 contests and five of the initiators went on to win. Designated winners were more likely to initiate primary contests against smaller opponents than were designated losers against larger opponents ($G_1 = 14.86$, $P < 0.001$). Prior contest experience (win, loss, or control; regardless of designation) had no significant effect on whether an individual initiated the secondary contest ($G_2 = 0.86$, $P = 0.65$). Initiators of the primary contest (winners or losers) were not more likely to initiate ($G_1 = 0.25$, $P = 0.62$) or win ($G_1 = 0.62$, $P = 0.43$) the secondary contest compared to individuals that did not initiate. Regardless of experience, focal individuals that initiated the secondary contest were more likely to win than noninitiators ($G_1 = 8.31$, $P = 0.003$).

Individuals were considered to have escalated a contest when they performed one or more high-risk behaviours against their opponent ([Supplementary Table S1](#)). Individuals (pooled designated winner and designated loser) that escalated the primary contest were more likely to win that contest than individuals that did not escalate ($G_1 = 21.48$, $P < 0.001$). Designated winners were more likely to escalate the primary contest against smaller opponents than designated losers against larger opponents ($G_1 = 5.42$, $P = 0.02$). Regardless of experience, escalating a primary contest did not influence an individual's probability of escalating the secondary contest ($G_1 = 0.98$, $P = 0.32$). Primary contest winners were more likely to escalate the secondary contest compared to primary losers ($G_1 = 4.3$, $P = 0.04$) and controls ($G_1 = 3.67$, $P = 0.05$). Individuals that escalated the primary contest, regardless of experience (win or loss), had a greater probability of victory in secondary contests than individuals that did not escalate the primary contest ($G_1 = 9.22$, $P = 0.002$). Regardless of prior experience, individuals that escalated the secondary contest were more likely to win the secondary contest than individuals that failed to escalate ($G_1 = 4.01$, $P = 0.05$).

Contest Duration

We calculated contest duration as the time between initiation and first retreat. Linear regressions were run using the masses of eventual winners versus eventual losers, masses of the larger individuals versus smaller individuals and mass asymmetries as predictors of contest duration in designated winner or designated loser primary contests. Our first analysis examined whether the mass of eventual winners/losers predicted contest duration. Linear regressions revealed no significant effect of eventual winner/loser mass on contest duration (Table 3). Similar results were seen when separate regressions were run for designated loser and designated winner contests (Supplementary Tables S2, S3). Our second analysis examined whether the mass of the larger/smaller individual predicted contest duration. Linear regressions revealed a significant effect of individual mass (larger versus smaller) on contest duration (Table 3). Contest duration decreased as a function of the larger individuals' mass but increased as a function of the smaller individuals' mass. These same trends held true when examining designated loser and designated winner contests separately (Supplementary Tables S2, S3). The full regression model indicated a significant negative relationship between mass asymmetries (winner–loser or larger–smaller) and contest duration (Table 3, Supplementary Tables S2, S3).

Metabolic Physiology and Behaviour

We provide the results from the MANOVA, both the full and univariate models, in Supplementary Table S4. Below we detail only the most pertinent information from those models, the results of the planned comparisons after sequential Dunn–Sidak adjustments, and results from pairwise correlations.

Plasma glucose concentration was the only metabolic measure to show a significant response to primary contest experience and treatment ($F_{8,155} = 3.29$, $P = 0.0003$). In treatment 1, primary contest winners had significantly higher plasma glucose concentrations than losers ($F_{1,155} = 19.64$, $P < 0.001$; Fig. 1) and controls ($F_{1,155} = 11.28$, $P = 0.001$; Fig. 1). Primary contest winners in treatment 1 also had significantly higher plasma glucose concentrations

Table 3
Linear regression modelling the effects of contest type, mass of winner/loser or larger/smaller, and their interaction on contest duration

	Estimate±SE	F	P	df
Full model: winner vs loser				
Absolute mass: overall model	—	1.06	0.39	5
Contest type	−68.44±41.62	2.70	0.10	1
Mass winner	92.24±49.18	3.52	0.06	1
Mass loser	59.84±46.85	1.63	0.20	1
Contest type×mass winner	26.16±49.18	0.28	0.60	1
Contest type×mass loser	−8.19±46.85	0.03	0.86	1
Full model: larger vs smaller				
Absolute mass: overall model	—	3.72	0.004*	5
Contest type	−58.04±38.99	2.21	0.14	1
Mass larger	−163.29±74.07	4.86	0.029*	1
Mass smaller	377.29±90.17	17.51	<0.001*	1
Contest type×mass larger	18.04±74.07	0.06	0.77	1
Contest type×mass smaller	−26.79±90.17	0.09	0.59	1
Full model: mass asymmetries (same for winner vs loser and larger vs smaller)				
Mass asymmetry: overall model	—	2.80	0.044*	3
Contest type	−6.93±36.26	0.04	0.85	1
Mass asymmetry	−208.61±74.43	7.86	0.006*	1
Contest type×mass asymmetry	−3.81±74.43	0.003	0.96	1

Estimates and their standard errors are provided; positive estimates indicate a positive relationship between mass (or mass asymmetry) and contest duration while negative estimates indicate a negative relationship between mass (or mass asymmetry) and contest duration.

* Denotes significance.

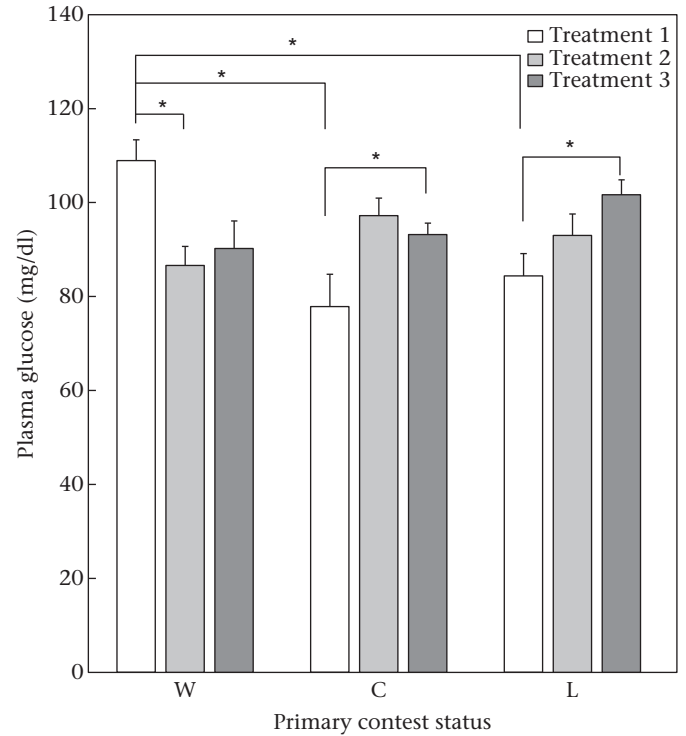


Figure 1. Plasma glucose concentrations in winners (W), losers (L) and controls (C) across all three treatments. Focal individuals were either processed immediately following the primary contest (treatment 1), processed 2 days following the primary contest (treatment 2), or processed following the secondary contest (treatment 3). * $P \leq 0.05$.

than primary contest winners in treatment 2 ($F_{1,155} = 10.46$, $P = 0.0015$; Fig. 1). Plasma glucose concentrations for winners in treatment 2 did not differ significantly from those of losers ($F_{1,155} = 2.51$, $P = 0.11$; Fig. 1) or controls ($F_{1,155} = 0.91$, $P = 0.34$; Fig. 1). Losers in treatment 1 had significantly lower plasma glucose concentrations than losers in treatment 3 ($F_{1,155} = 5.76$, $P = 0.0176$; Fig. 1). Controls in treatments 1 and 3 showed a similar trend ($F_{1,155} = 7.21$, $P = 0.008$; Fig. 1). Plasma glucose declined significantly as a function of presettlement high-risk behaviours in primary contest losers ($F_{1,14} = 5.92$, $r^2 = 0.33$, $P = 0.03$; Fig. 2) but not in primary contest winners ($F_{1,17} = 0.08$, $r^2 = 0.005$, $P = 0.78$; Fig. 2) in treatment 1. All other correlations between contest behaviour and plasma glucose were nonsignificant ($P > 0.05$) for both primary and secondary contests.

Liver lactate and muscle lactate showed no response to prior contest experiences ($F_{8,163} = 0.9$, $P = 0.52$; $F_{8,163} = 0.57$, $P = 0.80$; Supplementary Table S4, Figs S1, S2). However, in treatment 1, muscle lactate increased significantly in winners that performed more presettlement, low-risk behaviours ($F_{1,14} = 7.56$, $r^2 = 0.34$, $P = 0.015$; Fig. 3) but showed no such relationship for losers ($F_{1,17} = 0.001$, $r^2 = 0.001$, $P = 0.98$; Fig. 3). All other correlations between behaviour, muscle lactate and liver lactate were nonsignificant ($P > 0.05$) for both primary and secondary contests.

DISCUSSION

Our study demonstrates that prior losing experiences in male green anole lizards significantly decrease the probability of winning contests against size-matched opponents 2 days later. No noticeable winner effect was detected during the same time interval, at least with respect to the probability of winning. Loser

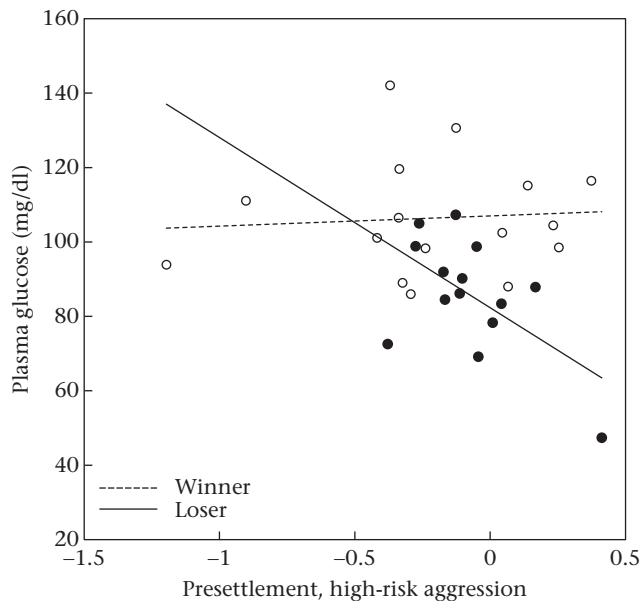


Figure 2. Plasma glucose concentrations in winners and losers in treatment 1 as a function of the frequency of presettlement, high-risk aggressive acts. Winner: dashed line, open circles; losers: solid line, closed circles.

effects were pronounced for nonescalated secondary contests (i.e. those that concluded without the use of escalated high-risk behaviours), which is strong evidence that losing experiences alter perceived fighting ability (Hsu et al., 2006, 2009; Hsu & Wolf, 2001; Kasumovic et al., 2010). Too few escalated contests were observed to determine, based on winning probabilities, whether prior contest experiences altered actual fighting ability. The lack of significant associations between one axis of resource-holding

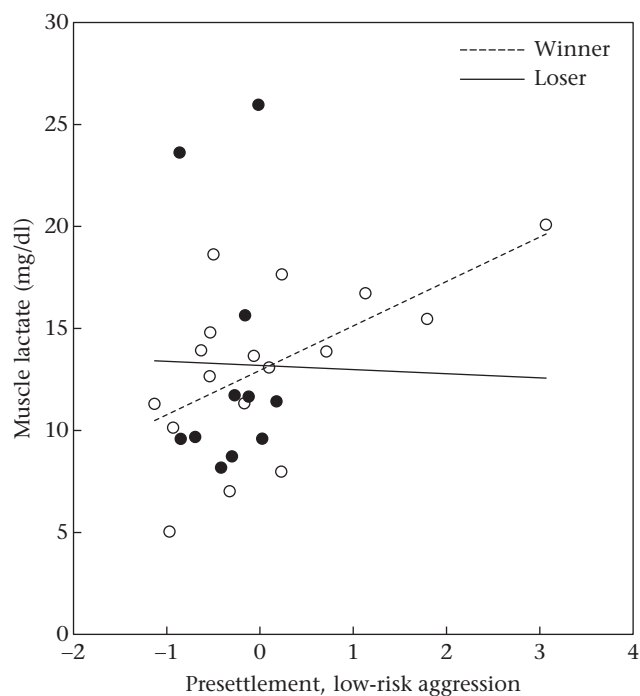


Figure 3. Muscle lactate concentrations in winners and losers in treatment 1 as a function of the frequency of presettlement, low-risk aggressive acts. Winner: dashed line, open circles; losers: solid line, closed circles.

potential (metabolic physiology) and secondary contest outcomes, however, suggests that winner and loser effects are not strongly driven by changes in actual fighting ability. For example, the loser effect was observed in the absence of any noticeable difference in metabolic physiology among winners, losers and controls 2 days following the primary contest (treatment 2). In fact, primary contest losers showed recovery of plasma glucose concentrations 2 days following their primary contest, as evidenced by the incremental rise in plasma glucose concentrations from animals that were euthanized immediately following primary contests (treatment 1) to animals that were euthanized following secondary contests (treatment 3). Because both losers and inexperienced controls showed the same pattern of glucose recovery, it is reasonable to conclude that an individual's actual fighting ability or resource-holding potential, as determined by their metabolic physiology (Briffa & Sneddon, 2007; Copeland et al., 2010; Ros et al., 2006), had not been altered solely as a result of a prior losing experience. Furthermore, although winners showed a peak in plasma glucose immediately following the primary contest, there was no evidence of a winner effect. Taken together, these results tentatively reject the hypothesis that prior contest experiences in green anoles alter actual fighting ability, but they provide relatively strong support for the hypothesis that prior contest experiences influence perceived fighting ability. With that said, an individual's actual fighting ability is the culmination of metabolic physiology, endocrine status, size, age, motivation, and more (Arnott & Elwood, 2009; Jonart, Hill, & Badyaev, 2007; Lailvaux et al., 2004; Moore, Obbard, Reuter, West, & Cook, 2008), which leaves much that has yet to be addressed.

Theoretical models predict that individuals should use prior contest experiences to reassess their fighting ability; winners increase while loser decrease perceptions of their fighting ability (e.g. Mesterton-Gibbons, 1999). These predictions have garnered significant empirical support (Hsu et al., 2006, 2009; Hsu & Wolf, 2001) and have been formalized into what is known as the 'perceived ability' hypothesis (Hsu et al., 2006; Rutte et al., 2006). However, Kasumovic et al. (2010) revealed the potential for prior contest experiences to alter an individual's actual fighting ability in addition to how they perceive their abilities. Our results are more consistent with prior contest experiences influencing an individual's perception of their fighting ability, and not their actual fighting ability. We hypothesize that the incongruence between our results and those of Kasumovic et al. (2010) can be traced back to the time intervals between contests used in each study. With a short time interval between consecutive contests (e.g. 1–2 h; Kasumovic et al., 2010), an individual could be exhausted or experiencing other physiological changes (e.g. neuroendocrine; Summers, 2001) that could affect their actual fighting ability. If changes in physiology are correlated with short-term changes in contest performance and probability of success, then the by-product hypothesis might accurately explain the presence of short-lived experience effects. However, the by-product hypothesis would not be relevant for long-lived experience effects (e.g. copperheads; Schuett, 1997) that persist past any observed changes in physiology. For green anoles, encounter rates are sporadic and often occur between neighbouring territorial males (Jenssen et al., 1995). This may preclude green anoles from displaying the by-product effect because the time between contests may be long enough to allow recovery from exhaustion or neuroendocrine changes that affect actual fighting ability. Research that tracks physiological changes and experience effects along both finer and coarser timescales (e.g. hours, days, weeks) will be essential for addressing the validity of the by-product hypothesis.

Contest performance (initiation and escalation) was a significant predictor of contest success in both primary and secondary

contests. Combatants more willing to initiate and/or escalate were more likely to win relative to those that did not. In primary contests, designated winners were more likely to initiate, escalate and win against a smaller standard opponent than were designated losers against a larger standard opponent. In secondary contests, prior contest status (win or loss) had no effect on the likelihood of initiation but had a significant effect on the likelihood of escalation; prior winners were more likely to escalate relative to prior losers. Changes in aggression commonly result from prior contest experience, with prior winners increasing and prior losers decreasing their aggressiveness, respectively (Hsu et al., 2006; Rutte et al., 2006). This change in aggressiveness can manifest into changes in the probability of future contest success (i.e. winner and loser effects). Our results demonstrate that prior losers show decreased aggressiveness and an associated decrease in the probability of future contest success (loser effect). Prior winners showed signs of a winner effect, as evidenced by increased aggressiveness in the secondary contest, but this did not translate into an increased probability of future contest success. Prior research has shown that winner and loser effects can alter an individual's aggressive behaviour without influencing their probability of future contest success (Huang et al., 2010). Our results suggest that while no 'winner effect' was observed in terms of contest success, prior winners showed markedly increased aggressive motivation.

Primary contest performance, not just status, was correlated with the probability of future contest success. Regardless of primary contest outcome, designated winners and designated losers that escalated their primary contest were more likely to win their secondary contest. These results are similar to what we found in our previous study in which designated losers that escalated their primary contest enjoyed a significant increase in future contest success (Garcia et al., 2012). We are left with a few possibilities that could explain these results. Intrinsic differences in aggression could exist between individuals of the same size class, with some individuals being consistently more aggressive or more likely to escalate, which would result in an increased probability of victory in secondary contests regardless of size asymmetries and prior experiences (McEvoy, While, Sinn, & Wapstra, 2012). Alternatively, future contest success may depend as much, if not more, on the quality of prior experience as on the status achieved (Beaugrand & Goulet, 2000). Escalated fights (high-quality experiences) may provide individuals with more information regarding their actual fighting ability than nonescalated fights (low-quality experience) and may make reassessment of their abilities more accurate (Beaugrand & Goulet, 2000). Animals that escalated primary contests may have gained information about their ability to persist in contests successfully, which translated into enhanced performance in secondary contests.

Prior contest performance and status had significant, interactive effects on metabolic physiology. Primary winners that engaged their opponent with more low-risk displays showed elevated muscle lactate concentrations. Green anoles often rely on displays to settle contests (Henningsen & Irschick, 2012; Jenssen et al., 2005; McMann, 1993). In lizards, the use of these displays has been correlated with decreased competitive endurance and increased muscle lactate (Brandt, 2003). Lateral compressions, for example, may impede an individual's ability to respire, forcing it to rely on anaerobic pathways for energy production (Brandt, 2003). Similar to the side-blotched lizards, *Uta stansburiana*, used in Brandt's (2003) study, the green anole relies heavily on lateral displays during aggressive contests (Jenssen et al., 2005; McMann, 1993). As such, individuals may have had to display more vigorously and incur greater metabolic costs (elevated muscle lactate) in order to win. This also is supported by our finding that, on average, primary contest winners in treatment 1 showed elevated plasma glucose

levels, indicating that they mobilized more of their energy stores than eventual losers or controls. Plasma glucose concentrations decreased as a function of the frequency of high-risk escalated behaviours in eventual losers of the primary contests. Multiple studies have demonstrated that as contests intensify, contestants incur more metabolic costs (Brandt, 2003; Copeland et al., 2010; Neat, Taylor, & Huntingford, 1998; Sneddon, Taylor, & Huntingford, 1999). The rate at which metabolic costs accrue predicts contest outcomes (e.g. losers lose because they accrue metabolic costs faster than do winners; Briffa & Elwood, 2001; Copeland et al., 2010; Matsumasa & Murai, 2005). Our results suggest that eventual losers might accrue costs at a faster rate than eventual winners, particularly during escalated contests. The results of our metabolic physiology assays suggest that, for green anoles, contest outcome is dictated by both the relative amount of metabolic cost that the opponents sustain and their relative ability to persist in the face of these costs.

The loser effect found in this present study contradicts our previous study (Garcia et al., 2012), which did not detect a loser effect during a 2-day time interval in the same species. We suspect that the variation between studies is due to differences in the methods employed. Housing conditions can influence individual behaviour (Lewejohann et al., 2006), and size of the contest arena in our previous study was half the size of the arena used in this study. The limited size of our contest arenas in the first study may have (1) limited the use of long-distance displays, which green anoles use frequently during aggressive contests (Henningsen & Irschick, 2012; Lailvaux et al., 2004; Lailvaux & Irschick, 2007; McMann, 1993) or (2) forced individuals to compete even when they desired retreat (desperado effect: Elias, Botero, Andrade, Mason, & Kasumovic, 2010; Grafen, 1987). Indeed, post hoc comparisons of behavioural performance showed a marked reduction in the probability of escalation in primary and secondary contests in the present study compared to our previous study (heterogeneity G test: primary: $G_1 = 5.33$, $P = 0.02$; secondary: $G_1 = 8.77$, $P = 0.003$). This indicates that, in the present study, individuals were more likely to settle contests without escalation. Under natural conditions, territorial males often settle contests using displays and rarely use physical aggression (Jenssen et al., 1995). Reduced escalation in our current study suggests that the new contest arenas were of a size that promoted natural contest behaviours. An alternative explanation for the differences between the two studies relates to experimental timing. Our first study quantified experience effects in early March through May while the present study quantified experience effects from early July through September. The breeding season in green anoles begins in early March and concludes, at the latest, in early August (Jenssen et al., 1996; Jenssen & Nunez, 1998; Licht, 1971, 1973); our experiments (previous and present) thus spanned the anole breeding season. For many taxa, including the green anole, individual behaviour and physiology can vary significantly before, during and after a breeding season (Hirschenhauser & Oliveira, 2006; Husak, Irschick, Meyers, Lailvaux, & Moore, 2007; Jenssen, Lovorn, & Congdon, 2001; Wingfield, 2005; Wingfield, Hegner, Dufty, & Ball, 1990). This raises the possibility that the presence and/or persistence of experience effects may vary within the breeding season (e.g. early versus late) or with transitions between breeding and nonbreeding seasons.

We previously found that individuals used a mutual assessment strategy during nonescalated contests and a self-assessment strategy during escalated contests (Garcia et al., 2012). In the present study, a large proportion of primary contests ($\approx 72\%$; across all treatments) concluded without the use of high-risk escalated behaviours. Based on our previous findings and the reduction of escalation observed in the present study we would expect most

individuals to use mutual assessment rather than self-assessment. We found that contest duration decreased as mass asymmetries between competitors increased in all contest types. Furthermore, we found that contest duration increased with the absolute mass of the smaller opponent (designated loser and standard loser) but decreased with the absolute mass of the larger opponent (designated winner and standard winner). These findings indicate that individuals use a mutual assessment strategy (Taylor & Elwood, 2003). A study on mangrove rivulus fish, *Kryptolebias marmoratus*, demonstrated that individuals switch assessment strategies when contests transition from display tactics to physical aggression (Hsu, Lee, Chen, Yang, & Cheng, 2008). Our present and prior studies (Garcia et al., 2012) were not designed to address switching of assessment strategies when contests transition between non-escalated and escalated phases. However, our studies provide evidence that the type of assessment strategy that an individual uses may depend on contest intensity (see Mesterton-Gibbons & Heap, 2014 for recent theoretical treatments).

Prior contest experience can significantly affect an individual's future contest performance and probability of success (Hsu et al., 2006). Theory predicts that experience effects arise when individuals reassess their fighting abilities in light of recent experiences (Hsu et al., 2006; Mesterton-Gibbons, 1999; VanDoorn et al., 2003a, 2003b). These predictions garnered significant empirical support (Hsu et al., 2006, 2009; Hsu & Wolf, 2001), but other potential mechanisms (e.g. by-product hypothesis: Rutte et al., 2006) were proposed and have gained support as well (Kasumovic et al., 2010). Our study tested one of the proposed alternatives, the by-product hypothesis, in addition to examining a potential physiological mechanism underlying experience effects in green anole lizards. We found that plasma glucose changes in a status-dependent fashion and that both plasma glucose and muscle lactate change as a function of behavioural performance during primary contests. While green anoles showed behavioural changes in response to prior losing and winning experiences, those responses were not closely associated with changes in metabolic physiology, one important component of an individual's resource-holding potential. Our results fail to support the by-product hypothesis, but lend additional support for the possibility that animals adjust their perceived fighting ability in response to contest experiences.

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Supplementary Material

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