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Tick parasitism impairs contest behavior in the western fence lizard (*Sceloporus occidentalis*)

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Abstract

Parasites may impair host behavior in ways that reduce host fitness, especially when access to territories or mates becomes disrupted. Western fence lizards (*Sceloporus occidentalis*) are a key host to western blacklegged ticks (*Ixodes pacificus*). Males are highly aggressive during the mating season, competing with rivals through displays of color badges, pushups, and other behaviors. We hypothesized that experimental tick infestation diminishes the performance of male western fence lizards in intrasexual contests, via either blood loss, damage to sensory structures, or both. We infested adult males with larval ticks for 7 to 10 days, staged contests between infested and quality-matched control males, and measured their behavior in enclosure arenas. Infested lizards were less aggressive and exhibited decreased hematocrit, compared to non-infested animals. We found no relationships between aggression and either body size or blue ventral badge color traits, for either infested or uninfested males. There was also no effect of tick attachment location and hemoparasite infection on host contest behavior. This is the first demonstration of the impact of *I. pacificus* parasitism on intraspecific interactions of western fence lizards and suggests that tick infestation has substantial impacts on lizard fitness. Because *I. pacificus* rely heavily on these lizards for blood meals and dispersal, these impacts could also influence the abundance of ticks and the pathogens they vector.

Significance statement

Parasitism frequently impairs host behavior. We investigated the effect of blacklegged tick parasitism on western fence lizard contest behavior using a lab-based manipulation experiment. We demonstrated that tick infestation reduced lizard aggressiveness, which may lead to territory loss and reduced mating opportunities in the wild. We found no evidence that host body size or coloration are correlated with aggression. This study adds to a growing body of evidence that tick parasitism is costly for lizards, while demonstrating the usefulness of manipulation experiments to understand host-parasite interactions.

Introduction

In the complex relationship between host and parasite, one basic question can be surprisingly difficult to answer: how

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harmful is parasitism to the host (Lehmann 1993)? Parasitism often results in altered host behavior, thereby reducing host fitness, as in the case of both parasite manipulation of hosts and alterations that are secondary to the physiological and immunological effects of parasitism (reviews in Klein 2003; Dantzer et al. 2008; Lafferty and Shaw 2013; Herbison 2017). Shifts in the behavior of parasitized animals may also be adaptive for the host, serving to minimize damage (Hart 1988), prevent further infection (Freeland 1976; Borgia and Collis 1990), or reduce current reproductive effort in favor of immunity when future reproduction is likely (Forbes 1993; Schwenke et al. 2016). The difficulty of disentangling these effects complicates our understanding of the costs of parasitism and highlights the benefits of manipulation experiments (McElroy and de Buron 2014). Even when the host is the

beneficiary of a shift in behavior (e.g., Deen and Hutchison 2001), that response may come at the expense of other activities, including territorial defense and other aspects of mate acquisition (Hart 1988; Forbes 1993; Dantzer et al. 2008). Altered host behavior in these contexts can be an evolutionarily important cost of parasite infection (Kortet et al. 2010).

Some key models for the evolution of sexually selected traits rely on the honesty of those traits as indicators of condition or some other measures of quality (Zahavi 1975; Johnstone 1995). The Hamilton-Zuk model integrates this logic with host-parasite evolutionary dynamics, suggesting that sexually selected traits reveal an individual's genetic ability either to avoid parasitism or to mitigate the detrimental effects of parasitism (Hamilton and Zuk 1982; reviews in Clayton 1991; Møller et al. 1999; Balenger and Zuk 2014). Much of this work has focused on coloration traits, which likely reflect a trade-off with immune defense (e.g., Borgia and Collis 1990; Megía-Palma et al. 2016a, b; Stephenson et al. 2020). However, behavior may be an even more sensitive indicator of parasitism because it can change more rapidly than most morphological traits (Folstad and Karter 1992; Wingfield et al. 1998; but see Møller et al. 1999). Parasitized animals that do not appear to be impaired under less demanding circumstances may betray their infection during agonistic interactions (Schall and Dearing 1987), and this can upend social hierarchies and disrupt access to mates (Rau 1984; Maksimowich and Mathis 2000).

Ectoparasites present unique challenges to their hosts, altering coloration (Torio 1992), taxing resources (Musante et al. 2007), and changing host behavior (Binning et al. 2013), but in ways that may differ from endoparasites. By obscuring or interfering with sense organs, ectoparasites can impair olfaction (Maksimowich and Mathis 2000), vision (Borucinska et al. 1998), and feeding (Brain and Bohrmann 1992), all of which may also adversely impact behavior. Inflammation beneath attachment sites may decrease the performance of underlying muscle, and hence locomotor performance (reviewed in Binning et al. 2018). Hematophagous ectoparasites can extract a sufficient quantity of blood to induce anemia (Salvador et al. 1996; Musante et al. 2007). Ectoparasites frequently suppress host immune responses to facilitate their own feeding, producing a vulnerability that can be exploited by pathogens (reviewed in Schoeler and Wikel 2001). Ectoparasites may also be visible to conspecifics, who may avoid interacting with infested individuals to reduce the risk of becoming infested, further impacting host fitness (Freeland 1976; Rosenqvist and Johansson 1995; reviewed in Clayton 1991).

Sceloporus (Phrynosomatidae) is a genus of small lizards in which the males of most species possess blue ventral badges (Ossip-Drahos et al. 2016) and defend semi-exclusive territories that most likely function in mate acquisition (Sheldahl and Martins 2000; Haenel et al. 2003).

Sceloporus occidentalis is common in many parts of western North America, with peak territoriality in spring and summer (Stebbins 2003). Aggressive behaviors, which are performed primarily by males, include pushup displays and lateral flattening, both of which present the normally hidden, sexually dimorphic ventral color badge to potential rivals (Sheldahl and Martins 2000). Although we know of no study investigating the role of this badge in contests between male *S. occidentalis*, a primary function of this badge in other sceloporines is rapid sex recognition by males during social interactions (Cooper and Burns 1987; Swierk and Langkilde 2013; Ossip-Drahos et al. 2018). Males also display to females in the wild (Cooper and Burns 1987), though little is known about female choice in this genus (but see Swierk et al. 2012). The vibrant blue-black color of the badge is produced via a combination of structural and pigment-based coloration: iridophores containing guanine crystal vesicles are arranged such that they reflect blue wavelengths (Morrison and Frost-Mason 1991), and melanophores basal to the iridophores absorb non-reflected wavelengths (Morrison et al. 1995; Quinn and Hews 2003). Although understudied, badges of this type may be energetically costly to maintain (Doucet and Meadows 2009; de Lanuza et al. 2014). Melanin-based coloration is generally linked via pleiotropy to a variety of traits that may enhance or constrain physiological and behavioral responses to parasitism (reviews in Ducrest et al. 2008; San José and Roulin 2018). Curiously, in *S. occidentalis*, there is scant evidence for the relationship among chest melanization, ectoparasite burden, aggression, and testosterone that has been observed in many other vertebrates (Seddon and Hews 2016, 2018).

The western fence lizard is parasitized by the western blacklegged tick (*Ixodes pacificus*) in many regions of California and the far Western United States (Furman and Loomis 1984; Clover and Lane 1995). Subadult *I. pacificus* (larvae and nymphs) take blood meals from a range of vertebrates including *S. occidentalis*, and nymphs especially infest these lizards frequently (Eisen et al. 2004). The relationship between *S. occidentalis* and *I. pacificus* is well known for its impact on the ecology of Lyme disease in the Western United States (Salkeld and Lane 2010; Swei et al. 2011). *S. occidentalis* possesses an innate defense against *Borrelia burgdorferi*, the bacterium responsible for Lyme disease (Burgdorfer et al. 1982), purging the pathogen from both tick and lizard tissues (Lane and Quistad 1998; Kuo et al. 2000). Despite the considerable interest in this association, we know of only one study addressing the effects of tick infestation on the behavior of *S. occidentalis* (Megía-Palma et al. 2020), and none has experimentally manipulated tick infestation to examine the impact on interactions with conspecifics.

Infestation with *I. pacificus* may impair male *S. occidentalis* fitness by reducing performance in aggressive contests. Tick infestation causes *S. occidentalis* to seek out cooler temperatures and lose body mass (Megía-Palma et al.

2020), both of which are indicative of a sickness response (Deen and Hutchison 2001). Tick attachment causes scarring and hyperkeratosis on lizards (Goldberg and Bursey 1991), which may be detrimental to aggressive behavior if it occurs on vital sensory structures. *I. pacificus* tend to attach in the nuchal pockets, folds in the dermis on the lateral part of lizard necks (Lane and Loye 1989). These pockets may be attractive to ticks because they provide shelter from harsh abiotic features of the environment and also because they contain soft scales; indeed, nuchal pockets may have evolved to divert ectoparasites away from sensory organs such as eyelids and tympanic membranes (Arnold 1986). Therefore, tick attachment outside of the nuchal pockets may reduce lizard fitness; for example, reduced host home range size is associated with tick attachment outside of similar pockets in other lizard species (Salvador et al. 1999).

Tick infestation may also exacerbate the detrimental impacts of a common hemoparasite of western fence lizards, *Plasmodium mexicanum* (phylum Apicomplexa, a malaria parasite; Ayala 1970; Schall et al. 1982). Lizards coinfected with both ticks and *P. mexicanum* have poorer body condition than those with only one of these parasites (Dunlap and Mathies 1993), and infection with *P. mexicanum* is negatively correlated with male *S. occidentalis* dominance status (Schall and Dearing 1987). Ticks and *P. mexicanum* both reduce host hematocrit (% cell volume in blood; Schall et al. 1982; Dunlap and Mathies 1993). Lizard territorial displays are aerobically demanding (Brandt 2003), so changes in oxygen carrying capacity, as might result from a reduction in erythrocytes, will likely manifest as altered behavior. *S. occidentalis* is infected with additional hemoparasites, including *Schellackia* (the species that infects *S. occidentalis* was recently reassigned to *Lankesterella* (Megía-Palma et al. 2017), and is here referred to as *Sch. (L.) occidentalis* to avoid confusion) and *Acroeimeria* (both phylum Apicomplexa), though a negative impact from these parasites has yet to be demonstrated (Megía-Palma et al. 2018).

We examined male *S. occidentalis* contest behavior following experimental infestation with lab-reared larval *I. pacificus* (after removal of attached ticks already present at the time of capture). We hypothesized that tick infestation reduces the aggressive behavior of male lizards by reducing their physiological capacities and/or obstructing their sense organs. This led to several predictions: (1) male *S. occidentalis* experimentally infested with ticks will exhibit less aggressive and more submissive behavior than size- and color-matched control animals during staged contests; (2) experimentally infested animals will exhibit reduced hematocrit compared to controls; (3) there will be a negative correlation between aggressive behavior and the number of ticks attached outside of the nuchal pocket (including around sensory structures). Some animals in our study were naturally infected with *P. mexicanum* and *Sch. (L.) occidentalis*, and we predicted that (4) there will be a negative correlation between infection with these hemoparasites and aggression.

Methods

Overview

Adult male (i.e., at least 60 mm snout-vent length (SVL); e.g., Schall and Sarni 1987) *S. occidentalis* were collected in spring and early summer and housed in isolation in outdoor enclosures. On the day of capture, we collected blood samples for hemoparasite detection and hematocrit measurement. Approximately 1 week after capture, we photographed the blue ventral badge to measure its size and color traits. Males were then paired based on collection date (such that differences in time in captivity were minimized), collection location (individuals were not paired with others collected close enough to have been familiar with each other in the wild), body size, blue ventral badge area, and tail status. After pairing, one male in each pair was infested with ticks. Following infestation or sham infestation, pairs of males were held for approximately 1 week prior to their use in trials, with males in a pair separated by an opaque partition at either end of an outdoor arena. After 1 week in these arenas (i.e., after 3 weeks in captivity), we removed the partition and video recorded behavioral interactions between paired lizards. Within 4 h of the conclusion of each trial, we removed and counted ticks and remeasured hematocrit. We used the blood collected earlier to accomplish hemoparasite screening, after the conclusion of behavior trials.

Generation of larval ticks for lizard infestations

To generate larval ticks for use in this study, we collected adult *I. pacificus* by dragging flannel flags over vegetation in livestock pastures, oak woodlands, and riparian areas in San Luis Obispo County, CA, December 2016 through January 2017. Adult ticks (23 males, 21 females) were fed to engorgement on cattle inside a confinement device (a modified cotton hat affixed with tag cement to the barrel of the cow) at the Beef Unit at California Polytechnic State University, San Luis Obispo (Cal Poly). Engorged female ticks were held in the laboratory inside mesh-covered scintillation vials partially filled with a mixture of activated charcoal and plaster of Paris during the oviposition period. Vials were held at 23 °C and 16:8 (light:dark) cycles inside desiccators containing sterile water in the bottom to maintain ~100% humidity, preventing desiccation and encouraging rapid egg development (Pollock et al. 2012). Larvae hatched approximately 40 days after oviposition and remained in these vials until used for experimental infestations of lizards.

Lizard collection and processing

During the seasonal breeding period (April–July), we collected 100 adult male *S. occidentalis* by noose on the Cal Poly campus. Collection locations spanned an approximately 5-km radius, including ornamental plantings in the core campus, green spaces among student housing, oak woodlands, and riparian areas. Lizards bearing signs of previous collections (clipped digits) were excluded from this study. Within a few hours of capture, we transported the animals to the lab in cloth bags and housed them in outdoor semi-enclosures, as described below. On the day of capture, we clipped the digits of each lizard in a unique pattern for individual identification. Lizard mass was measured to the nearest 0.5 g with a spring scale (Pesola AG, Schindellegi, Switzerland). Also on the day of capture, blood (60–90 μL) was collected from the right infraorbital sinus with a heparinized microhematocrit tube (Fisher Scientific, Chino, CA, USA) to determine hematocrit and identify lizards infected with *P. mexicanum* and *Sch.* (*L.*) *occidentalis* (see below). A small amount of blood was diverted for hemoparasite screening, with the remainder centrifuged for 5 min at 10,000 rpm in a microhematocrit centrifuge to determine hematocrit. On the same day, we thoroughly inspected the surface of each lizard under a dissecting microscope (Olympus SZ60) at × 60 magnification and removed all wild ticks with forceps. Of the 100 animals captured for this study, 49 had wild ticks on them, and the mean ± SE number of these wild ticks per lizard was 5.1 ± 1.1.

Photography of badges

We quantified the area, hue, saturation, and brightness of each lizard's blue abdominal badge and measured the jaw width (a likely correlate of bite force, and therefore dominance status, in some lizard species; Huyghe et al. 2005), using digital images taken within 2 weeks of capture. To minimize the effects of lighting and temperature variation, all photographs were obtained at a single station in a temperature-controlled (25 ± 1 °C) room. A Nikon D800 camera (Nikon, Japan) set to obtain images in NEF format was mounted on a tripod in the center of four full-spectrum (5000K) LED lamps, which were bolted to the corners of a 72 × 58.4 cm flat-white painted stage (see Wojan et al. 2019 for an image of the setup). These lamps provided omnidirectional lighting. During photography, we held lizards by their snout and tail base, photographing their venter (for badge measurements) and dorsum (for jaw width) in rapid succession. Handling time per lizard was less than 1 min. Each image included a ruler and photography standard with color targets and a grayscale.

Image files were converted to TIF format without compression, imported into Fiji (Schneider et al. 2012; Schindelin et al. 2012) and converted to HSB stacks, from which we extracted hue, saturation, and brightness values using the "Polygon" tool to select the left blue ventral badge (not including any bordering black or partially blue scales). We measured the mean value of this region in the hue, saturation, and brightness channels (Fig. S1). Measurements in each of the three channels were calibrated with the "Calibrate" tool, using the grayscale and color standard placed beside subjects (Hamilton et al. 2005). Blue badge area was measured by applying a color threshold that calculated the area occupied by blue pixels in the region between the armpits and the femoral pores. All morphological measurements were made by one person, prior to infestation and assignment of lizards to pairs. A summary of morphological variables is given in Table S1.

Enclosures and testing arenas

Lizards were housed in outdoor enclosures throughout this study, except the 48-h infestation period (see below). These enclosures also served as the arenas for staged contests. We constructed the enclosures from 203-L plastic storage bins (height 49.5 cm, width 54.25 cm, length 116.5 cm; Fig. S2A). Animals were housed individually in 447-cm^2 subdivisions within these enclosures prior to infestation/sham infestation and in 1343-cm^2 subdivisions in the same enclosures afterwards. After approximately 1 week, these enclosures served as 2686-cm^2 arenas for video-recorded contests, following the removal of opaque plastic partitions separating paired animals (see below). We filled the arenas with white aquarium gravel to a depth of approximately 1 cm, to permit burrowing. We draped shade-cloth lengthwise over half of each arena, which cast a patch of partial shade on the substrate and allowed for thermoregulation. Each compartment also contained a hide made of clay. The interior of the arena was painted white to reduce temperature extremes and improve contrast in video recordings. We oriented the arenas such that each side received approximately equal sunlight, especially during behavior trials held in the afternoon. Each lizard was offered 2–3 crickets (*Acheta domesticus*) daily. The sides of the arenas were heavily misted with water during feedings, as lizards occasionally drank droplets from the arena walls. We erected silvered tarps over enclosures on days when temperatures exceeded 35 °C, or when rain was forecasted. Enclosures were elevated approximately 20 cm above the ground, which served to further reduce temperatures and decreased the risk of flooding.

Pairing and infestation

To maximize the parity between lizards within each pair, we paired lizards that were collected (and hence had their first blood draw) within a few days of each other (mean difference ± SE = 1.52 ± 0.29 day) and that were photographed on the same day. We paired males on the

basis of tail autotomy status (i.e., lizards with broken tails were only paired with each other), SVL (0.78 ± 0.08 mm), mass (1.08 ± 0.13 g), and the proportion of the ventral surface occupied by blue scales ($5.8 \pm 0.6\%$), with priority given to pairing criteria in that order. We used a random number generator to assign individuals within each pair to infested and control treatments. Two-sided permutation tests (R package: "coin"; function: "independence_test"; Hothorn et al. 2006; with tests stratified by lizard pair) confirmed that infested and control lizards did not differ significantly in SVL ($Z = 0.73$, $P = 0.47$), mass ($Z = -0.2$, $P = 0.84$), jaw width ($Z = -0.99$, $P = 0.32$), ventral blue badge area ($Z = -1.56$, $P = 0.12$), number of wild ticks ($Z = -1.24$, $P = 0.21$), ventral badge hue ($Z = 0.11$, $P = 0.91$), saturation ($Z = -1.05$, $P = 0.29$), brightness ($Z = 0.29$, $P = 0.77$), or initial hematocrit ($Z = 0.26$, $P = 0.80$). Although hemoparasite infection status was unknown until after the behavioral trials, paired permutation tests revealed no significant difference in *Sch.* (*L.*) *occidentalis* infection rates between treatment groups ($Z = -0.63$, $P = 0.53$, seven and five infected animals assigned at random to infested and control treatments, respectively). Only a single lizard in each treatment group was naturally infected with *P. mexicanum*, and this was therefore dropped from further analysis.

We infested lizards with larvae in late spring and early summer, when larval ticks were between 38 and 119 days post-eclosion. Within 24 h of a planned infestation, motile larvae were chilled to facilitate handling and individually counted ($n = 100$) into microcentrifuge tubes using a stereoscopic microscope and paintbrush. This is a large but ecologically relevant number of ticks for this population of lizards: for example, Lumbad et al. (2011) report a spring tick burden of 15 to 130 per male (including both larvae and nymphs). Lizards were infested with ticks in 2.8-L glass beakers, above which we positioned 50-W reptile basking lights (Zoo Med Laboratories, Inc., San Luis Obispo, CA), and around which we wrapped paper to maintain visual isolation between lizards. This ensured that males were unfamiliar with each other prior to trials. We exposed each lizard to the 100 larvae, first by gently tapping the tubes to dispense larvae across the dorsal surface of the animal, then by taping tubes to the beaker walls to permit any remaining larvae to leave the tube. We placed mesh tops on beakers to contain ticks during an infestation period of 48 h. Control animals were exposed to identical conditions, minus the ticks. After 48 h, we removed the lizards and placed them in the arena enclosures. The arena occupied by the pair and the side given to each member of the pair were assigned at random. To ensure that lizards were infested for the duration of the behavioral trials, we counted the number of ticks on each lizard at the conclusion of each trial, as described below.

Staged contests

We tested a total of 50 pairs of lizards in interactions staged in outdoor enclosures in late spring and summer (mid-May through early August 2017). Each pair and individual were tested once. The lizards in each pair were acclimated in the arenas for a mean of 7 days (SE = 0.11 days), to resume typical behaviors after exposure to a novel environment and permit attached larvae to be established in the mid to late feeding phase (Pittman et al. 2013) to evaluate parasitism impacts. Trials lasted for 30 min, were held between 13:50 and 17:00, and were video recorded using surveillance cameras (Lorex, Markham, ON, Canada) mounted on tripods positioned at either end of the arena prior to testing (Fig. S2C). As lizards appeared to be disturbed by the camera placement, we waited for 10 min after camera placement before removing the partition to initiate the trial. Partitions were removed with a long pole that kept the observer out of view. With the partition removed, a slit cut in the shade-cloth that had accommodated the partition cast a sun fleck in the middle of the arena at the new border of the lizard "territories"; we intended this to motivate contests between lizards, similar to centrally located basking lights employed in other studies (e.g., Garland et al. 1990; Huyghe et al. 2005; Fig. S2B; Online Video 1). We monitored trials in real time to ensure that lizards were neither seriously harmed nor escaped from the arenas, although behaviors were scored from the video recordings. At the end of each trial, we recorded the temperature using a thermometer inserted into the substrate of the partially shaded region in the middle of the arena. Each arena was used several times over the 3-month study period, and we minimized any odor cues left by previous occupants by washing arena interiors with soap and water and also by pooling and mixing substrate from all arenas during cleaning events after each group of trials.

Immediately following the trials, we removed lizards from the arenas and quantified tick intensity and attachment location. Ticks were removed with forceps with the aid of a stereo microscope. Attachment location was recorded using these categories: eyelid, external auditory meatus, nuchal pocket, limb, or "other" (vent, tail, ventral and dorsal scales). On the same day as the trial, we also drew a second blood sample for hematocrit determination, following the same procedure described above. Although unlikely, to verify that ticks had not transferred between animals during the acclimation period or trial, we inspected both the infested and control lizards for ticks. To ensure a complete tick count, we held post-trial lizards for 1 week in wire cages above Fluon (polytetrafluoroethylene)-coated, water-filled trays in a 23 °C incubator, with a 16:8 (light:dark) photoperiod, to collect any larvae missed during post-trial inspections. Ticks recovered in these trays were categorized as having attached to the "other" body region location category, though only a few ticks were recovered in this way. We euthanized post-trial lizards by

inducing deep anesthesia with isoflurane, followed by decapitation.

Behavioral data collection

Video files were imported into BORIS v.6.3.8 (Friard and Gamba 2016) to score behaviors. We created an ethogram consisting of twenty-five behaviors, seven of which (scratch, twitch, hide, kick, startle, wrestle, and perch) were extremely rare and were therefore excluded from further analysis, leaving us with 18 behaviors analyzed (escape attempt, chemosensory, reposition, vigilant (non-basking), burrowed, retreat, vigilant (basking), flattened (basking), exploring, exploring, flattened (non-basking), approach, charge, full-show, two-leg pushup, four-leg pushup, bites/nudges, shudders; Table S2). We viewed each trial video twice, once to score the behavior of each animal (order within and across pairs determined randomly). Most videos were reviewed by at least two people (of four total reviewers) working together. To minimize observer bias, blinded methods were used when all behavior data were scored. The video resolution was also too low for the reviewers to inadvertently determine the status by seeing ticks. Because of potential effects on behavior, we did not conspicuously mark individuals. Instead, video reviewers tracked individuals visually using the subdivision within the arena as the starting point, and employing slow-motion playback (sometimes frame-by-frame) when animals moved quickly or were in close proximity to each other. The animals spent the majority of the trial time far apart from each other, making them easily distinguishable using these methods. If observers lost track of an animal, data erroneously attributed to the other animal were repeated in the records of both, and we reviewed trial data and rectified such errors.

Hemoparasite screening

We screened blood drawn from lizards upon capture for *P. mexicanum* and *Sch. (L.) occidentalis*. Tests for *P. mexicanum* included both scans of blood films and nested PCR, whereas only blood films were used to screen for *Sch. (L.) occidentalis*. Blood films were prepared and stained in a protocol similar to procedures described in Schall (1983) and Eisen et al. (2001). In brief, we fixed dried blood films in 100% methanol for 1 min, then stained the films in 10% Giemsa (JT Baker, Avantor Performance Materials, PA, USA) prepared using pH 7.0–7.2 phosphate-buffered solution for 50 min. Slides were scanned for at least 3 min for the presence of intraerythrocytic stages of *Sch. (L.) occidentalis* and *P. mexicanum*. *P. mexicanum* gametocytes were distinguished from *Sch. (L.) occidentalis* sporozoites by the displacement of host erythrocyte nuclei, which can be seen in

erythrocytes infected with *P. mexicanum* but not *Sch. (L.) occidentalis*, and by the equatorially banded nucleus in *Sch. (L.) occidentalis* sporozoites (Bonorris and Ball 1955; Ayala 1970; Megía-Palma et al. 2014; Fig. S3). *Plasmodium mexicanum* trophozoites, schizonts, and merozoites, which also develop in *S. occidentalis* peripheral blood, were also searched for in this screening (Ayala 1970; Schall 1982).

We used nested PCR to verify the presence or absence of *P. mexicanum* from blood samples, as this method can effectively detect very low parasitemia infections (Vardo et al. 2005). Genomic DNA was extracted from dried lizard blood dots on filter paper taken during blood draws for initial hematocrit determination (DNeasy Blood and Tissue Kit, Qiagen, Hilden, Germany), using the manufacturer-provided genomic DNA extraction protocol for nucleated blood samples. We used primers and reaction conditions described in Vardo et al. (2005) to amplify a 673-bp region of the *Plasmodium* cytochrome *b* gene (Table S3). Each set of PCR reactions included a positive control (DNA extracted from *P. mexicanum*–infected *S. occidentalis* blood samples provided by J. Schall, Department of Biology, University of Vermont) and a negative control (ultrafiltered water in lieu of DNA template). We used 1 μL of the template in each round, with DNA extracted from lizard blood samples as a template for the outer reaction, and products of the outer reaction as a template for the inner reaction. Specificity of the primers for *P. mexicanum* was verified using blood samples from a pair of infected and control *Sceloporus undulatus* provided by S. Bailey and W. Clark at Western Wyoming Community College. The presence of 673-bp *P. mexicanum* cytochrome b amplicons was determined by running PCR products on 1.5% agarose gels (Fig. S4).

Data analysis

To reduce the number of morphological variables and uncover latent factors, we applied factor analysis (R package: "psych"; functions: "fa," "fa.parallel"; Table 1; Revelle 2018) to these variables and used the factor scores in further analyses. We retained two varimax-rotated factors using standard factor analytic criteria and interpreted these based on variables with |loadings| > 0.4 (Costello and Osborne 2005) on each factor, as follows: factor 1 body and badge size, which included SVL, mass, jaw width, and blue ventral badge area, and factor 2 badge size and color, which included badge area, saturation, and hue (with lower and higher values corresponding to red and blue portions of the color wheel, respectively; Yasir and Qin 2009). Infested and control animals did not differ significantly in either factor 1 (paired t test: $t = 0.76$, $P = 0.45$) or factor 2 ($t = 0.88$, $P = 0.38$), although the standard deviation in pair differences was higher for factor 2 (SD = 0.93) than factor 1 (SD = 0.50).

Table 1   Results of the factor analysis on morphological variables

Morphological variable	Factor 1 Body and badge size	Factor 2 Badge size and color
SVL	*0.90*	−0.09
Mass	*0.95*	−0.04
Jaw width	*0.70*	−0.18
Blue abdominal area	*0.56*	*0.64*
Blue badge hue	−0.21	*0.76*
Blue badge saturation	0.03	*0.85*
Blue badge brightness	−0.10	0.34
Eigenvalue	2.57	1.86
Percent variance explained	36.7	26.6
Cumulative percent variance explained	36.7	63.3

Loadings used to interpret factors are in italics

A second factor analysis was used to reduce the number of behavior variables and uncover latent factors. We first used a Kaiser-Meyer-Olkin test (Kaiser and Rice 1974; R package: "psych"; function: "KMO"; Revelle 2018) to determine which behaviors had been sampled adequately. Of the 25 behaviors in the ethogram, 11 had individual measures of sampling adequacy (MSAi) ≥ 0.6, indicating that they were suitable for factor analysis (Kaiser and Rice 1974). Applying the same factor analytic criteria used in our factor analysis on morphological variables, we again retained two factors, which we interpreted based on variables with |loadings| > 0.4, as follows: factor 1 aggressiveness, which positively correlated with the frequency or duration of behaviors that are widely interpreted as signifying aggression (pushups,

shudders, approaches, and charges; Garland et al. 1990; Sheldahl and Martins 2000; Seddon and Hews 2016) and factor 2 reposition-escape, which positively correlated with the number of small movements, escape attempts, bites-nudges, and approaches (Table 2).

We used a stratified permutation test (R Package: "coin"; function: "independence_test"; Hothorn et al. 2006) to test the prediction that tick infestation reduces hematocrit. Because there was a significant general decline in hematocrit, we compared the magnitude of the decrease in hematocrit between infested and control animals. Pair ID was entered as a block term. We used a permutation test rather than a *t* test because the residuals of changes in hematocrit were not normally distributed.

Table 2   Results of a factor analysis on lizard contest behaviors

Behavior	Factor 1 Aggressiveness	Factor 2 Reposition-escape
Contact (non-aggressive)	0.03	0.23
Escape attempt	−0.05	*0.53*
Chemosensory	0.12	*0.39*
Reposition	0.24	*0.76*
Full-show	0.34	0.15
Two-leg pushup	*0.79*	−0.03
Four-leg pushup	*0.45*	0.15
Bites/nudges	*0.42*	*0.44*
Charges	*0.53*	0.25
Approaches (slow)	*0.78*	*0.40*
Shudders	*0.78*	−0.22
Eigenvalue	3.08	1.18
Percent variance explained	0.25	0.14
Cumulative percent variance explained	0.25	0.39

Loadings used to interpret factors are in italics

We created a pair of general linear mixed models to address whether tick infestation and other variables influenced the behavior of lizards during trials (R package: "lme4"; Bates et al. 2015). Each model included scores on one of the behavior factors (i.e., factor 1 aggressiveness or factor 2 reposition-escape) as the response variable, with tick treatment, temperature, factor 1 body and badge size, factor 2 badge size and color, the intensity of wild ticks on lizards at initial collection, and *Sch. (L.) occidentalis* infection as first-order predictors. We included enclosure temperature because it was our closest proxy for body temperature, and body temperature is important for male *S. occidentalis* contest behavior (Engbretson and Livezey 1972). Before fitting the final model, we fit models with all possible two-way interactions between predictors and iteratively re-fit these models until only significant interactions remained. We used an α value of 0.05 for determining which interactions to include. In both models, this process resulted in the retention of only first-order terms. Both models included pair ID as a random effect. Factor 1 aggressiveness was $\log(+1)$ transformed to most closely approximate normality of residuals, as assessed with the Shapiro-Wilk test (R package: "stats"; function: "shapiro.test"; R Core Team 2018; $W = 0.96$, $P = 0.01$). The response variable in the factor 2 reposition-escape model required no transformation (Shapiro-Wilk: $W = 0.98$, $P = 0.11$).

To evaluate the effects of tick attachment site on behavior, we fit a second pair of linear models for the two behavior factors (factor 1 aggressiveness and factor 2 reposition-escape) exclusively among infested animals. These models included the proportion of ticks that attached in the nuchal pocket as a predictor. Other predictors were shared with the previously described models, including factor 1 body and badge size, factor 2 badge size and color, and temperature. Just as with the first pair of models, we determined which interactions to include by first fitting models with all two-way interactions, then performing a manual backward selection based on significance ($\alpha = 0.05$). After arriving at the final model, we applied a Bonferroni correction to the results of the four behavior models, yielding an α value corrected of 0.0125.

To explore whether infested and control animals differed in the temporal sequence of behaviors, we created (first-order) Markov chains from behavioral observations. These depicted the probability of one behavior following another and provided a general indication of how specific patterns of behavior may have differed between infested and control animals. We included two-leg pushups, slow approaches, four-leg pushups, charges, bites/nudges, full-shows, and retreats in this part of the analysis, as these behaviors have been classified as "aggressive" or "submissive" in previous studies of *S. occidentalis* (Schall and Dearing 1987; Garland et al. 1990; Sheldahl and Martins 2000; Seddon and Hews 2016, 2018). Retreat behavior was inadequately sampled for factor

analysis (see above) but was included in the Markov chain because of its historical inclusion in studies of *S. occidentalis* contest behavior (e.g., Garland et al. 1990). The information contained in the Markov matrices allowed us to identify patterns of behavioral differences between infested and control animals, but we did not subject these to statistical analysis because their purpose was to provide context for the results of the mixed effects models.

Results

Tick-infested lizards had significantly lower scores on factor 1 aggressiveness than control lizards ($F_{1,48} = 7.16$, $P = 0.010$; Table 3, Fig. 1). Infested animals had lower aggression scores than non-infested animals in 30 of 50 pairs (Fig. S5). Factor 1 aggressiveness increased with temperature ($F_{1,46} = 10.98$, $P = 0.002$; Fig. S6). No other terms were significantly correlated with factor 1 aggressiveness after correcting for multiple tests (all other $P > 0.15$). There were also no significant predictors in our model for factor 2 reposition-escape (all $P > 0.013$), although there was a marginally non-significant trend towards males with higher scores on factor 2 badge size and color scoring higher on factor 2 reposition-escape in both the general version of this model and the model including tick attachment location, suggesting that larger and more colorful males made more small movements and escape attempts.

In 40 of 50 pairs, both lizards engaged in aggressive behaviors, as inferred from descriptions of such behaviors in other studies (see above; Table S2). On average, lizards spent approximately 12% of each trial (3.6 min) displaying, approaching, or retreating (Fig. S7). The temporal sequence of agonistic behaviors differed between infested and control animals (Fig. 2, Table S4). Most notably, after retreating, control animals were far more likely to re-approach the opponent, whereas infested animals were more likely to assume a static full-show posture. Infested animals were more likely to retreat following full-shows than control animals. After a series of pushups, control animals were also more likely to approach opponents than infested animals. These differences in behavior complement the general conclusion that infested animals behaved less aggressively than their non-infested opponents.

The mean tick intensity (number of attached ticks) post trial was $68.82 \pm SE = 3.39$ (range 13–99, $N = 50$). Ticks attached in the nuchal pocket far more frequently than any other location (Fig. 3). Aside from the marginally non-significant correlation mentioned above, there were no significant relationships between the proportion of ticks that attached inside the nuchal pockets and lizard behavior (all $P > 0.013$).

Hematocrit declined significantly between capture and behavior trials for both control and infested animals (paired t tests: $t = 7.16$, $df = 49$, $P < 0.001$, and $t = 12.11$, $df = 49$, $P < 0.001$, respectively; Fig. 4). However, the reduction was

Table 3 Relationship between predictors and factor 1 aggressiveness

	Estimate (SE)	F	df residual	P
Tick infestation status	*0.37 (0.14)*	*7.16*	*48.16*	*0.010*
Temperature	*0.05 (0.02)*	*10.98*	*46.65*	*0.002*
Factor 1 body and badge size	–	1.04	53.18	0.312
Factor 2 badge size and color	–	2.07	76.40	0.154
Tick intensity at collection	–	0.56	92.90	0.457
Sch. (*L.*) *occidentalis* infection	–	0.67	92.81	0.415

Estimate and SE for the tick infestation variable is for control lizards. The P and F values are taken from type III Wald F tests on the model output. Significant effects are indicated in italics ($\alpha = 0.0125$)

significantly greater for infested animals (two-sided permutation test; $Z = 3.34$, $P < 0.001$; R package: "coin"; function: "independence_test"; Hothorn et al. 2006).

Discussion

Aggressiveness is an important determinant of contest outcome in sceloporine lizards (Garland et al. 1990; Robbins et al. 2010). We demonstrated that male *S. occidentalis* lizards infested with *I. pacificus* ticks were less aggressive in staged contests, less likely than control animals to re-approach a rival following a retreat, and more likely to retreat after a full-show display. Both the agonistic behaviors and their durations were similar to those seen in natural settings (Carpenter 1978; Schall and Sarni 1987; Sheldahl and Martins 2000; Haenel et al. 2003; Seddon and Hews 2016). Because the main benefit of territoriality for male sceloporines appears to be access to

Fig. 1 Effects plot from the linear mixed-effects model showing the relationship between factor 1 aggressiveness and tick treatment. Bars span the 95% confidence interval

females (Haenel et al. 2003), it is likely that tick infestation decreases the reproductive success of males by reducing their ability to defend territories in the wild. Schall and Sarni (1987) report that subordinate, *Plasmodium*-infected *S. occidentalis* perform fewer displays to females than dominant animals. Male lizards expose themselves to increased tick infestation as a result of expanded territorial patrols during the breeding season (Olsson et al. 2000; Pollock et al. 2012; Wieczorek et al. 2020), and our findings suggest an additional way in which maintaining these territories may be costly. Our results also suggest that when in the breeding season lizards are infested may further impact fitness; males infested early in the breeding season are likely to suffer a greater reduction in mating success due to reduced territorial behavior than males infested later in the breeding season, when territories are well established (Stamps and Krishnan 1998).

The proximate mediator for reduced aggression in parasitized lizards may be a reduction in oxygen carrying capacity (Brandt 2003), as suggested by the greater decline in hematocrit among infested animals compared to controls. This finding supports previous studies demonstrating negative effects of *I. pacificus* on other aspects of *S. occidentalis* physiology (Dunlap and Mathies 1993; Megía-Palma et al. 2020). Diminished locomotor performance could be responsible for the behavioral effects we observed, as a 25% decrease in hemoglobin corresponds to a 20% reduction in stamina (Schall and Sarni 1987). The dynamics of tick infestation are likely to vary temporally and spatially, and the tick burden we administered was high. However, it was well within the range for male lizards in the wild during the spring peak of subadult tick activity (Lumbad et al. 2011). Furthermore, we infested the lizards with larvae only, whereas in the wild, they may also harbor the much larger nymphs, which are likely to result in far greater blood volume loss (Dunlap and Mathies 1993). Therefore, the challenge we delivered is realistic, and if it did reduce the aerobic capacity of *S. occidentalis* via reductions in blood oxygen carrying capacity, this would echo the impact of high parasitemia *P. mexicanum* infections in *S. occidentalis* (Schall 1982; Scholnick et al. 2010).
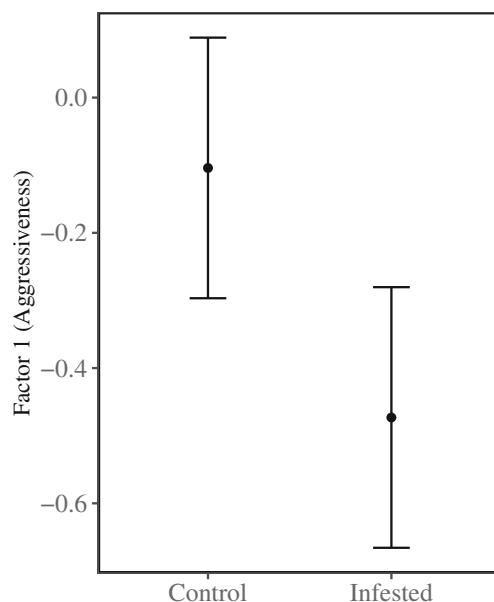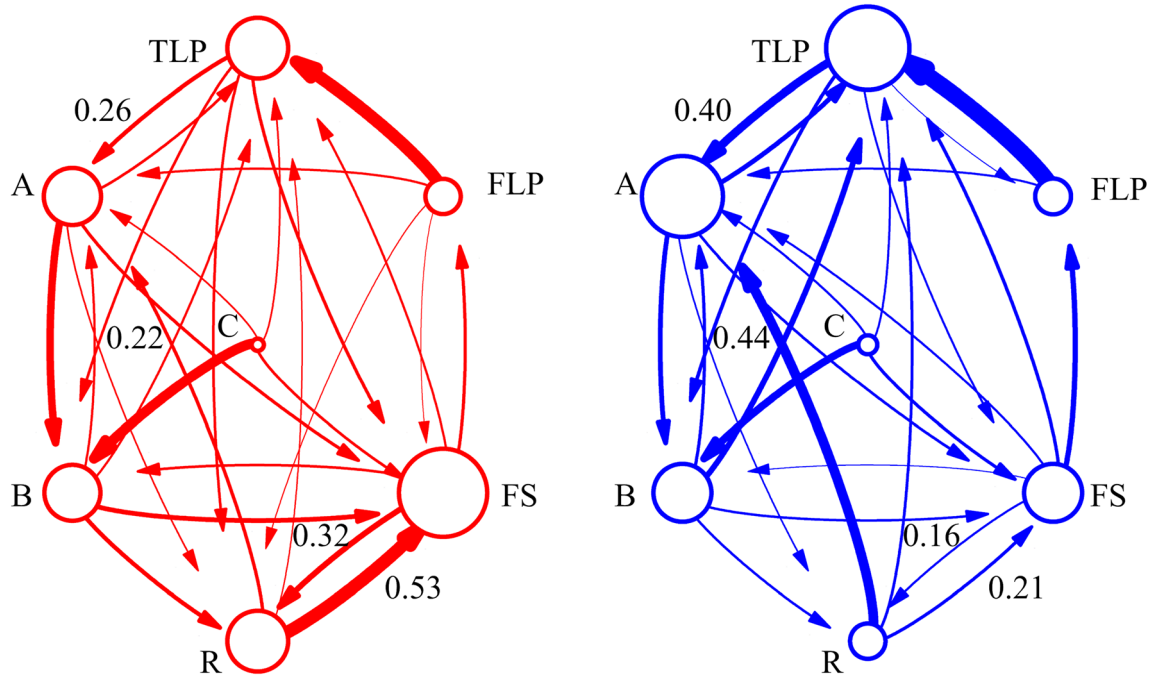
Fig. 2 Diagram of a Markov chain created for selected agonistic behaviors, including two-leg pushups (TLP), four-leg pushups (FLP), full-show starts (FS), retreats (R), bites/nudges (B), approaches (A), and charges (C), exhibited by infested animals (left) and control animals (right). Arrows depict the transition frequency between these behaviors, with arrow thickness proportional to this frequency. Transition frequencies for R → FS, FS → R, R → A, and TLP → A appear numerically next to the corresponding arrows (see Table S3 for the full transition matrix). The diameter of each node is directly proportional to the proportion of that behavior out of the total counts of the 7 behaviors depicted for each treatment. For clarity, transition frequencies below 0.1 are not drawn. Transitions to repeat behaviors were discarded, primarily because pushups occur in highly repetitive sets

Lizards may gather information about an opponent's fighting ability using body size (Stamps and Krishnan 1994) and badge characteristics (Olsson 1994; Berglund et al. 1996; Huyghe et al. 2005). Because we size matched opponents, it is not surprising that the unavoidable, small differences in body size between males within pairs did not influence aggression. However, we did not match the males as closely with respect to badge traits (hue, saturation, brightness, and badge size); given that full-shows reveal badges to rivals, it was somewhat surprising that this behavior was not a significant component of our measure of aggressiveness, and that there was no relationship between badge traits and aggressiveness, regardless of infestation status. However, these findings are not unique; Seddon and Hews (2016, 2018) found no relationship between aggressiveness and chest melanization within populations of *S. occidentalis* (though they did find a negative relationship across populations that differ in elevation). The role of badges in agonistic behavior in the closely related (and morphologically similar) *S. undulatus* has been more extensively studied. In this species, ventral abdominal coloration seems to play a role in sex recognition by males at the beginning of social interactions, but males may use other traits when deciding whether to escalate agonistic interactions (Cooper and Burns 1987; Ossip-Drahos et al. 2018). Our study broadly concurs with previous work in this genus in that badge characteristics were not more important than other factors, in this case tick infestation, in predicting aggression. As we only measured coloration prior to infestation, future studies could address whether *S. occidentalis* color badges are altered by tick infestation.

The positive correlation between temperature and aggressive behavior we observed conforms to the trend expected for reptiles, as they are selected to elevate body temperature during social interactions to optimize physiological performance (Regal 1971; Engbretson and Livezey 1972; Martin and Huey 2008). Although we measured substrate temperature and not body temperature, Megía-Palma et al. (2020) found that the preferred environmental temperature of *S. occidentalis* is negatively correlated with tick load. Given this previously reported relationship, and our finding that external temperature positively correlates with aggression, it seems probable that lower body temperature in infested animals explains at least part of their reduced aggressiveness relative to control animals. Temperatures in arenas rarely (four trials) exceeded the preferred resting temperature of *S. occidentalis* (34 °C). Male *S. occidentalis* body temperatures reach as high as 40 °C during agonistic encounters (Engbretson and Livezey 1972). Curiously, temperature did not correlate significantly with small movements and escape attempts, even though
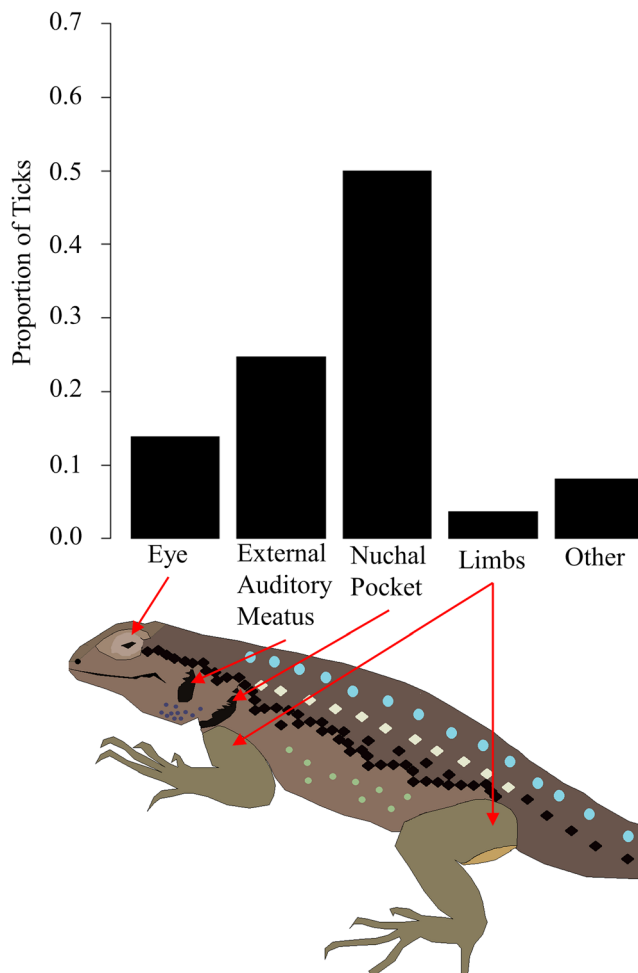
Fig. 3 The proportion of tick larvae that attached at each location. Shaded regions on the lizard represent the extent of each of the defined locations. Ticks found on unshaded regions were classified as "other"
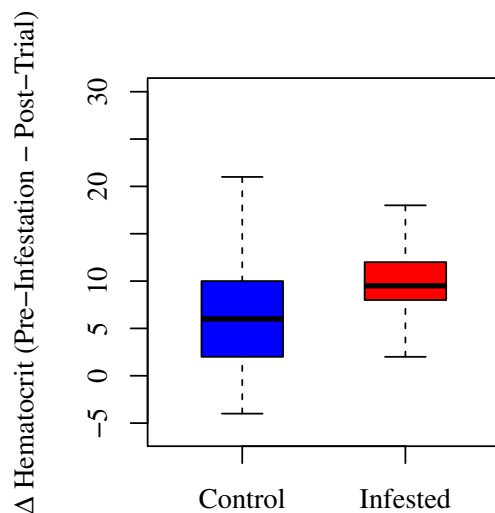
Fig. 4 Change (initial value minus final value) in hematocrit between capture and the conclusion of trials for infested and control *S. occidentalis*. Solid lines within boxes represent the median, boxes extend from the 25th to the 75th percentiles, and whiskers extend to extreme values that are within 3/2 of the interquartile range beneath the 25th and above the 75th percentiles (more extreme outliers are excluded)

these are behaviors that would seem to demand considerable energy expenditure.

The impact of tick-induced hematocrit reduction on lizard aggression may be even more pronounced than we demonstrated. For example, although the intensity of ticks on our animals (approximately 68 per male) was within the range of 15 to 130 reported in field studies of the same population of *S. occidentalis* (Lumbad et al. 2011), in the field in late spring, larvae are outnumbered by nymphs (Eisen et al. 2001), which are far larger than larvae and ingest considerably more blood (Dunlap and Mathies 1993). Because our study was limited to larvae to simplify tick rearing and analysis, the significant effect of tick infestation we report may actually underestimate the true impact of subadult *I. pacificus* on *S. occidentalis* in the wild.

We found no significant relationship between the proportion of ticks attached outside the nuchal pocket and either aggressiveness or reposition-escape behavior. Because most ticks that did not attach in the nuchal pocket attached to the eyelid and external auditory meatus, this implies either that tick attachment did not disrupt the senses sufficiently enough to impact these behaviors, or that disruption of these senses by ticks may not affect lizard contest behavior. Our findings therefore do not support the hypothesis that nuchal pockets evolved to divert ectoparasites from sensitive structures in the context of aggression (Arnold 1986). These pockets could be spandrels (Gould and Lewontin 1979) in *S. occidentalis*, though there are alternatives to the methods we employed that have supported an adaptive function for these structures in other species (e.g., Salvador et al. 1999). Sensory obstruction by ticks may affect lizard fitness outside of short-term aggressive contexts, such as by rendering lizards less able to patrol territories, or more vulnerable to predators. We also cannot discount that larger nymphal ticks, which also attach to western fence lizards, may exert such an effect. Finally, the benefit ticks may derive from attaching in pockets, such as shelter from abiotic conditions or grooming, should be explored in greater detail.

The proportion of animals naturally infected with the apicomplexan hemoparasites *Sch.* (*L.*) *occidentalis* and *P. mexicanum* was far below seasonal peaks reported in other California *S. occidentalis* populations (Ayala 1970; Schall et al. 1982; Megía-Palma et al. 2018). It may be that our study population is infected at a rate higher than our sample suggests; much of our work was conducted during the spring, whereas *P. mexicanum* infections peak in the summer, and even at its peak, the spatial distribution of infections is patchy (Schall 1982). *P. mexicanum* has a broad range of effects on *S. occidentalis* physiology (Schall et al. 1982), coloration (Ressel and Schall 1989), and behavior (Schall and Dearing 1987; Schall and Houle 1992), and these effects may be amplified when combined with tick infestation (Dunlap and Mathies 1993). By contrast, no effect of *Sch.* (*L.*) *occidentalis*

infection on *S. occidentalis* has yet been demonstrated (Megía-Palma et al. 2018). We found no correlation between *Sch. (L.) occidentalis* infection and behavior based on the 12 lizards infected with this parasite. This may be explained by an overall reduced pathological impact of *Sch. (L.) occidentalis* on *S. occidentalis* as compared to *P. mexicanum* (e.g., Megía-Palma et al. 2020), but future studies will be necessary to test this idea. Additionally, these lizards harbored mites, and possibly other ectoparasites and endoparasites (e.g., Goldberg et al. 1998; Megía-Palma et al. 2018). It would be interesting to examine the potential synergistic effects of such infestations and infections on aggression, in future studies.

Our results may have ecosystem-level effects if reduced host aggression resulting from tick infestation has subsequent effects on tick distribution and abundance, as *S. occidentalis* is a vital host for *I. pacificus* (Swei et al. 2011). This tick species relies on hosts for dispersal over distances of more than a few meters (Lane et al. 2010), so the dispersal of ticks depends to a certain degree on the lizards on which they feed. If reduced aggressiveness leads to smaller territories for infested lizards, ticks may suffer reduced opportunity for dispersal and decreased access to preferred microhabitats. However, ticks may also benefit if, for instance, infested lizards spend less time on prominent basking perches (where ticks may be exposed to desiccating conditions), as appears to be the case among lizards infected with *P. mexicanum* (Schall and Sarni 1987). *I. pacificus* vectors several pathogens relevant to human health (Brown and Lane 1992; Eshoo et al. 2015), and our study demonstrates an aspect of the interaction of this parasite with a critical host that deserves further investigation.

Parasite-induced asymmetry could be used to investigate decision making in animal conflicts. Assessment strategies during contests, including the capacity to integrate information about self relative to opponent, have been studied in many species (Arnott and Elwood 2009; Fawcett and Mowles 2013), including lizards (Martin et al. 2016). A key feature of ectoparasites is that they are easily detected at close range, so it is possible that the lizards can see ticks on their opponents, and that the aggressiveness disparity we observed was in part due to control animals' increasing aggressiveness against an opponent they perceived to have diminished resource holding potential. Future work, including studies employing sham infestation with artificial ticks, could advance our understanding of such effects. Additionally, although our paper is focused on male-male interactions, female mate choice is an important determinant of male reproductive fitness, even in highly territorial species (e.g., Kamath and Losos 2018). Male *Sceloporus* lizards display to females in the wild (Cooper and Burns 1987), and females exhibit preference for traits, including some that are associated with intrasexual dominance (Swierk et al. 2012). Although little is known about mate choice in *S. occidentalis* specifically,

observations of females interacting with males during and after male-male contests may indicate a close link between intrasexual contests and female choice in this species (Schall and Dearing 1987). It is plausible that tick infestation is detrimental to courtship displays in addition to contest behavior, which would represent a second way in which infestation could impact host fitness. This idea also deserves future study.

Declarations

Ethics approval and consent to participate All applicable international, national, and/or institutional guidelines for the use of animals were followed. Use of animals was approved by the California Polytechnic State University, San Luis Obispo, Institutional Animal Care and Use Committee (protocol #1609), and a California Department of Fish and Wildlife permit (#SC-013426 to DL). Consent to participate is not applicable.

Consent for publication All authors have reviewed the manuscript and consent for publication

Conflict of interest The authors declare that they have no conflict of interest.

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