Seeing orange: prawns tap into a pre-existing sensory bias of the Trinidadian guppy
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Sensory bias, a predisposition towards certain signals, has been implicated in the origin of mate preferences in some species. A risk associated with these biases is that they can be co-opted by predators as sensory lures. Here we propose that the orange spots on the brown pincers of a diurnal, predatory species of prawn function as lures for Trinidadian guppies, which have a sensory bias for orange. We exposed female guppies to (i) a life-like model of this Trinidadian prawn with orange, green or no spots on the pincers or (ii) a live, novel (non-Trinidadian) crustacean (crayfish), also with spotted pincers. First, we provide evidence that guppies sympatric with the prawn recognized our model as a potential predator. Next, we found that guppies spent more time in the dangerous head region of the model prawn with orange-spotted pincers compared with unspotted pincers. Finally, we show that allopatric, but not sympatric, guppies spent more time in the vicinity of the head of a live crayfish when orange spots were added to its pincers than when brown spots were added. Our results suggest that the orange spots on prawn pincers can act as a sensory lure.

Keywords: sensory bias; mate choice; predator–prey; Poecilia reticulata; Macrobrachium crenulatum; visual lure

1. INTRODUCTION

In order to effectively detect and acquire resources, animals are often tuned to respond to specific types of stimuli, such as particular motions or colours [1]. There is mounting evidence that females’ pre-existing sensory biases can be co-opted by males and that this can contribute to the origin of female mate preferences [2–4]. Although sensory biases can be beneficial, for example through enhanced resource detection, these biases can be co-opted by predators as sensory lures [3,5]. Sensory lures are colour patterns, behaviours or other signals that predators use to manipulate the behaviour of potential prey [6,7].

Prey-specific chemical lures used by predators are quite diverse and well studied [8]; for example, bolas spiders, Mastophora hutchinsoni, mimic the sex pheromones of female moths to lure male moths [9]. In contrast, the use of visual cues by predators to lure their prey has not been studied explicitly in many systems; in most of these cases, predators attract prey by mimicking their prey’s food (e.g. angler fish [10]; mussels mimic minnows [11]) or habitat [12], such as the yellow stripes of the orb-web spider Argyope bruennichi, which are believed to mimic flowers [13]; the preference of bees for flowers with highly contrasting petal coloration is exploited by Thomisus spectabilis, a predatory crab spider whose contrasting colour pattern increases bee visitation rates to flowers on which it is stationed [14]. In the case where predators mimic potential mates, female fireflies of the genus Photuris lure and prey upon heterospecific males by mimicking the flashes that the males’ conspecific females use as a mating signal ([15,16], reviewed in [8]). However, to the best of our knowledge, there are no documented cases where the predator co-opts a visual sensory bias that is both a foraging and a mating signal in the prey species. Here, we ask whether an invertebrate predator is using a sensory lure to attract a vertebrate prey via a sensory bias that is involved in both mating and non-mating contexts.

The Trinidadian guppy, Poecilia reticulata, is a model system for the study of mate preference evolution (reviewed in [17–19]). Mature males display multi-coloured patterns to females and the males with large, intense patches of orange tend to be the most attractive to females, although the strength of the orange preference varies among populations [20,21]. Sensory bias has been implicated in the origin of this preference for orange; both males and females are attracted to orange objects outside of a mating context, possibly as a foraging adaptation (e.g. for finding orange-coloured fruit [22,23]).

Over part of their natural range in Trinidad, guppies co-occur with an invertebrate predator, a diurnal prawn, Macrobrachium crenulatum [24,25]. At those sites, adult prawns are an inconspicuous brown colour except for an obvious area of orange at the joint between the dactyl and the propodus of their pincers (chelae) (see electronic supplementary material, figure S1a). Because the prawns are thought to be insensitive to orange [24,26], it seems unlikely that these patches of orange are for intraspecific communication. Indeed, it has been assumed that the high levels of orange on male guppies in diurnal prawn
sites results from relaxed natural selection on this trait by prawns, which has allowed a private communication channel for guppies [24,27,28]. We have observed prawns, in both the field and the laboratory, partially hidden with their pincers open and extended outward, seemingly waiting for prey to approach. There are several lines of evidence that this species is a predator of guppies: prawns are known to prey on guppies under laboratory conditions (A.C.P., personal observation [29]), guppy scales have been found in the guts of wild-caught prawns [19], fin damage and mortality rates in wild populations are consistent with predation by prawns [19,25], and there is an association between male guppy coloration and prawn presence or abundance [24,28].

Given the guppy’s documented attraction to orange, we hypothesized that the prawn’s orange pincer spots lure guppies closer to the prawn’s dangerous claws. If the prawn and its orange spots impose significant selection on guppies, this could add a new layer of complexity to the evolution of guppy colour preferences in both mating and non-mating contexts. We tested the sensory lure hypothesis by asking whether guppies would be more likely to closely approach a prawn with orange-spotted pincers than a prawn with unspotted pincers. To control for variation in the behaviour of prawns, we first tested guppies with a naturally coloured model, cast from a preserved specimen, either with or without orange spots on its pincers. We reasoned that, if the orange spots are acting as a lure, guppies should direct more approaches towards and spend more time near the head region of orange-spotted models than the head region of unspotted models. To complement those results, we indirectly tested the lure hypothesis by comparing the responses of guppies from populations that co-occur with the diurnal prawn to those that do not. Guppies that co-occur with *M. crenulatum* are more wary of this species, avoiding the area around the pincers of live prawns and instead inspecting the less dangerous tail region, than guppies that do not co-occur with it [30]. Therefore, we predicted that sympatric guppies would be less susceptible to the proposed sensory lure than allopatric individuals; however, guppies from both population types should be more susceptible to prawns with orange-spotted claws than unspotted ones, given the guppies’ bias for the colour orange. For this experiment, we included a control model to allow us to verify that the guppies did indeed react to the prawn models as though they were live prawns, and to separate any population-based differences in exploratory behaviour [31] and neophobia from differences in predator inspection behaviour.

We also tested an alternative hypothesis: that these spots contribute to predatory success by obscuring the outline of the claws (disruptive coloration hypothesis). If this is the case, we expected guppies to approach and spend more time near the pincers of prawn models bearing spots of any contrasting colour, orange or not, compared with models without spots. We tested this by exposing a subset of guppies from both population types to prawn models with green spots on the pincers in place of orange spots.

In a second experiment, to determine whether orange-coloured spots would be effective as lures when used by a live predator, we examined the responses of guppies to a novel (non-Trinidadian) crayfish species. As with the first experiment, we manipulated the coloured spots on the pincers. We predicted that guppies would again spend more time near the head region once orange-coloured spots were added to the otherwise brown prawns; however, we predicted that the responses of sympatric and allopatric guppies might not be as distinct as in the first experiment, as all guppies were unfamiliar with this species of crayfish.

### 2. MATERIAL AND METHODS

#### (a) Experiment 1: the model prawn (*Macrobrachium crenulatum*)

The *M. crenulatum* specimen used to create the prawn models (electronic supplementary material, figure S1a,b) was collected from a tributary of the Paria River from the North Slope of Trinidad (see below for details). Based on photographs of live *M. crenulatum*, casts of the preserved prawn were painted a drab reddish-brown/black, with an area on each claw painted with an orange or green spot, or left unspotted (brown) (see the electronic supplementary material for details). Given guppy-specific estimates of perceived brightness (Pr) and chroma (Dmax) for these paints (calculated in Rodd et al. [23]), the green paint provided a brighter but approximately equally chromatic stimulus for comparison against the orange paint (green: P_r = 0.69, D_{max} = 0.93; orange: P_r = 0.50; D_{max} = 0.94). Using the same technique, a control model of roughly similar size and dimensions was also cast from Lego blocks. The control was included in experimental trials to ensure that the guppies’ responses to the prawn model were not caused by either neophobia or neophilia; for example, if a guppy avoided both the prawn and control models, this could indicate that the individual was fearful of novel objects. Spots of comparable size (to the prawn model) were added to the arbitrarily designated ‘anterior’ side of the control model. In any given trial, the colour of these spots was always identical to the spots on the prawn model.

Only adult females were tested in this experiment, as they are more likely to engage in predator inspection behaviour than males and juveniles [30] and because female responsiveness to orange is of particular interest given its relevance in both mating and non-mating contexts. The experimental guppies were lab-reared descendants of fish from four Trinidadian populations, two where guppies coexist with *M. crenulatum* (‘sympathetic’ populations) and two where they do not (‘allopatric’ populations); none of the fish had been previously exposed to prawns. In both ‘sympathetic’ populations, tributaries to the Marianne and Paria Rivers, which are on the north slope of the Northern Range, prawns are regularly encountered, sometimes at very high densities, and have been observed striking at nearby guppies (A.C.P. and F.H.R. 2005, 2008, personal observation). Both ‘sympathetic’ sites are ‘low’ predation sites where guppies also coexist with an omnivorous killifish, *Rivulus hartii*, which preys on juveniles and small adults [30,32]. ‘Allopatric’ populations were descended from ‘low’ and ‘high’ predation stretches of the Aripo River on the south slope of the Northern Range. ‘Low’ and ‘high’ predation sites both have *R. hartii*, but ‘high’ predation sites also include predatory cichlids and other dangerous piscivores [19].

Using a simultaneous-choice design, each fish (total n = 275) was presented with a prawn model and a control that were spaced approximately 30 cm apart in the left or right half of the experimental tank. Model placement (left or right side) and orientation (facing the front or back of the aquarium) were randomized for each trial. We visually
established columnar zones surrounding the models; the upper boundary of the zones was a line drawn on the tank 7 cm above the gravel and the outer perimeters of the zones were delimited by burying a clear plastic rimmed disc (6 cm radius) under the gravel beneath each of the models so that only the rim was visible to the observer. Each columnar zone was partitioned into halves, corresponding to the head and tail zone of each model (see electronic supplementary material, figure S2). At the beginning of each trial, one fish was placed in the experimental tank and observations were recorded once the guppy was active or after 1 min if the fish remained motionless at the start of the trial. Behaviours were recorded for 10 min using JWatcher (v. 1.0). We recorded the numbers and durations of approaches the female made into each of the four zones. We also recorded any pecks that the fish directed towards the models as this behaviour would surely put a guppy at risk of predation and this behaviour indicates obvious attraction towards the model.

(b) Experiment 2: live, novel crayfish

In the second experiment, guppies (total n = 94) from the same four populations were exposed to a non-Trinidadian crayfish. The experimental design and conditions were the same as those in the first experiment with the differences noted below. Three tropical crayfish (tentatively identified as Procambarus sp.) were obtained from Downtown Pets and Aquarium, Toronto, Ontario, Canada. Crayfish were roughly similar in size and shape to the M. crenulatum model used in the first experiment. One orange or brown spot (see electronic supplementary material, figure S3 for reflectance spectra) was painted on each of the crayfish’s pincers for approximately half of its trials; for the rest of the trials, spots were switched to the alternate colour to control for behavioural differences between individual crayfish. Given our findings in experiment 1 (described below) and in order to increase sample size for other spot colour treatments, we chose to not use green-spotted crayfish in this experiment. The crayfish responded to guppies in a predatory fashion; crayfish oriented towards guppies and often tracked their movement around the tank. On occasion, a crayfish attempted to grab a guppy with its pincers. Thus, these crayfish were suitable as potential predators that were unfamiliar to all Trinidadian guppies.

One day prior to experimental trials, a crayfish was placed in the experimental tank with a clay-pot shard (for shelter) and allowed to acclimate overnight. The following day, the shard was removed and the crayfish was given 30 min to settle before the trial commenced. An individual adult female was randomly selected from one of the four populations listed above and observations commenced once the guppy became active. Behaviours were recorded for 15 min using JWatcher. We recorded the numbers and durations of approaches by the guppy towards the crayfish head, middle and tail region. A guppy was considered to approach a crayfish if it entered a visually estimated region approximately 4 cm away from the crayfish. The crayfish’s behaviours were also noted (see the electronic supplementary material).

(c) Analyses

(i) Experiment 1: the model prawn (Macrobrachium crenulatum)

We analysed the total durations of visits to the model zones, rather than the numbers of visits, because duration should better represent the risk faced by the guppy from the ‘predator’. If orange spots act as lures, guppies should spend more time in the head region of orange-spotted than non-orange-spotted models. The few guppies that did not enter any zones during their trial were excluded from all analyses. We had intended to use a repeated-measures ANOVA on the raw data but were unable to do so because many guppies did not enter every zone resulting in zeros and thus violating an assumption of the test. Rather than use the less powerful Friedman’s test, we ranked the duration that each guppy spent in a specific zone (e.g. prawn head) compared with all other guppies in the experiment (i.e. each guppy received a rank from 1 to 275 for each zone) and analysed the ranks with a repeated-measures ANOVA with zones as the repeated measure [33]. Ties were given the average of the ranks. The numbers of pecks were also analysed by ranking individuals within a zone, for the 57 guppies that pecked at least one of the models.

We performed a separate analysis of the first zone that each guppy approached since it could provide valuable insight into what part of the prawn was the initial target of inspection, as well as providing another measure of risk faced by guppy. We predicted that guppies, especially allopatric ones, would be more likely to approach the head region before the tail region when the pincers were orange-spotted than unspotted. We used a replicated G-test for goodness-of-fit to determine whether spot colour or population of origin affected a guppy’s first approach to the prawn model.

In an attempt to simulate the fitness consequences of close approaches to the head of the prawn model, we ran survival analyses based on a proportional hazards model [34]. We expected allopatric guppies to be more vulnerable to the prawns, across all treatments, than sympatric ones. Based on our observations of the live crayfish (see below), we assumed that a prawn would catch or seriously injure a guppy that spent three or more seconds (consecutively, in one visit) in the prawn head zone, as this should place a guppy within striking distance of the prawn. In order to check the robustness of our results to this assumption, we repeated the survival analysis using one second as the minimum time required for the prawn to harm a guppy.

(ii) Experiment 2: live, novel crayfish

For the second experiment, there were again zeros for some individuals for some zones. Therefore, for each of the three crayfish zones separately, we ranked the durations that individual guppies spent in a zone. Ties were given the average of the ranks. As for the model prawn analysis, the ranked data were analysed with a repeated-measures ANOVA.

3. RESULTS

(a) Experiment 1: the model prawn (Macrobrachium crenulatum)

We performed several analyses to demonstrate that the sympatric guppies recognized the prawn model as a potential predator. First, we compared the duration spent with the unspotted control and unspotted prawn models. The few guppies that did not enter any zones during their trial were excluded from all analyses. We had intended to use a repeated-measures ANOVA on the raw data but were unable to do so because many guppies did not enter every zone resulting in zeros and thus violating an assumption of the test. Rather than use the less powerful Friedman’s test, we ranked the duration that each guppy spent in a specific zone (e.g. prawn head) compared with all other guppies in the experiment (i.e. each guppy received a rank from 1 to 275 for each zone) and analysed the ranks with a repeated-measures ANOVA with zones as the repeated measure [33]. Ties were given the average of the ranks. The numbers of pecks were also analysed by ranking individuals within a zone, for the 57 guppies that pecked at least one of the models.

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the sympatric guppies spent less time within the prawn head zone than any of the other three zones (prawn tail, control head and control tail) (see below; see electronic supplementary material, table S1 for a summary of responses to both models, and electronic supplementary material, table S2 for the numbers of guppies that approached the head zones of the prawn model and live crayfish). Next, we compared, for the unspotted prawn model, the ranks of the durations that guppies spent in the head zone with the time spent in the tail zone. Sympatric guppies spent significantly less time in the prawn model head zone than the tail zone (repeated-measures ANOVA, \( F_{1,67} = 26.35; p < 0.0001 \)), while allopatric guppies spent significantly more time in the head zone (\( F_{1,33} = 22.44; p < 0.0001 \)) than the tail zone (see electronic supplementary material, table S1 for medians and quartiles). Paired \( t \)-tests gave the same results for the comparison of the prawn models (results not shown). For the unspotted control models, neither group of guppies showed a significant difference in the durations they spent with the ‘head’ versus ‘tail’ zones (allopatric: \( t \)-ratio for paired \( t \)-test = \(-0.93\), d.f. = 33, \( p > 0.05 \); sympatric: \( t \)-ratio = \(0.90\), d.f. = 67, \( p > 0.05 \)). Overall, we interpret these results as indicating that sympatric guppies, but not allopatric guppies, recognized the prawn models as potentially dangerous predators.

We then used three metrics (durations spent in the zones, the zone that was first approached and the numbers of pecks) to ask whether (i) guppies might be more vulnerable to prawns with orange-spotted pincers than unspotted or green-spotted ones and (ii) whether allopatric guppies are especially vulnerable to these putative/candidate lures. We compared the ranked durations spent by sympatric and allopatric guppies in the head and tail zones of the prawn models with orange, green or spotless pincers. Because interaction terms between the main effects can be unreliable in analyses of ranked data [35,36], we broke the data set into six ‘treatment’ groups: two population types (allopatric, sympatric) with three treatment colours (unspotted, orange and green) to simplify the interpretation of these results. In the repeated measures ANOVA, there was a significant zone \( \times \) treatment interaction (\( F_{2,269} = 13.12; p < 0.0001 \)). To examine this interaction in more detail, we analysed the population types separately. For populations sympatric with the prawn, pincer spot colour had a significant effect on the responses to the prawn model (figure 1 and table 1); they spent significantly more time in the head zone of models with orange-spotted pincers than models with green or unspotted pincers. Allopatric guppies spent significantly more time near the model (head and tail) when spots were orange compared with when the pincers were green-spotted, with the response to unspotted pincers intermediate between the other two (figure 2). Overall, allopatric guppies spent more time in the head zone than the tail zone but, for these guppies, there was not a significant interaction between the zone and the colour. Allopatric guppies might be responsive to the head region of prawns simply because the head and pincer structures are more elaborate than the tail. We note that a caveat of using an ANOVA with ordinal data is that we could only determine if one ranked mean was significantly higher or lower than another ranked mean; the relative differences between the two means have to be interpreted with caution.

![Figure 1. Mean values of the ranked durations that guppies from allopatric and sympatric populations spent in the head and tail zones of the prawn model with orange, green or no spots added to the pincers. For each zone, all guppies in this experiment were ranked from lowest to highest duration spent in that zone and the mean rank was calculated for each treatment type, e.g. allopatric guppies tested with brown (unspotted pincers). For guppies from populations sympatric with the prawn, there was a significant zone \( \times \) colour interaction (repeated-measures ANOVA: \( F_{2,182} = 8.48, p < 0.0003 \)). For allopatric guppies, there was a significant colour effect (\( F_{2,87} = 4.08, p < 0.03 \)). Allopatric populations are denoted by solid lines; sympatric with dashed lines. The colour of each line indicates the colour of the spot on the pincers, with brown representing unspotted pincers.](http://rsb.royalsocietypublishing.org/Downloaded from)

<p>| Table 1. ANOVA table summarizing the between-subject (i.e. overall differences among individuals in responsiveness to pincers with orange, green or no spots) and within-subject (i.e. differences in responsiveness to the head versus tail zones and whether those differences vary with the colour of the spots on the pincers) effects of spot colour on the ranked durations of time spent in the head versus the tail zones of the prawn model for guppies from populations allopatric and sympatric with the diurnal prawn. |</p>
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As predicted, sympatric guppies avoided the head zone of the model prawn during their first approaches to the unspotted and green-spotted models (for both comparisons: \( G \)-value < 9.4, d.f. = 3, \( p < 0.05 \)), but for orange-spotted models, sympatric guppies did not avoid the prawn head zone during first approaches (orange: \( G \)-value = 0.29, d.f. = 3, \( p = 0.96 \)). In contrast, allopatric guppies did not differentiate between the zones for their first approaches...
Figure 2. ‘Survival’ analysis for sympatric and allopatric guppies in the prawn head zone with trials for all three spot colours combined. Results are based on a proportional hazards model (SAS Proc Phreg, SAS Institute Inc. [34]). We assumed that a prawn would catch a guppy that spent three or more seconds, consecutively, in the prawn head zone. In this scenario, the prawn would have captured half of the allopatric guppies by the end of a 600 s trial. Allopatric guppies were 2.8 times more likely to be ‘caught’ than sympatric guppies (p < 0.0001). The upper line represents sympatric populations; the lower line represents allopatric populations.

...for any of the three colours (heterogeneity G-value: 6.33, d.f. = 6, p = 0.78).

Only results for pecks of the head zone of the prawn model are presented here because they should put the guppy at greatest risk and because it is the location of the proposed ‘lure’. For this analysis, pecks at the head zone refer to pecks directed at both the head region and pincers of the model; however, the majority of pecks of the green- and orange-spotted models were directed towards the coloured spots on the pincers. Overall, there was not a significant difference in the number of times allopatric versus sympatric guppies pecked the head zone of the prawn model (F1,55 = 2.0, p = 0.16; electronic supplementary material, table S3) but the two groups varied in their responses to models with different coloured spots. Sympatric guppies were more likely to peck the head region of the orange-spotted prawn model than the unspotted model (F2,30 = 9.7, p = 0.0006); pecks of green-spotted models were intermediate between the other two. Allopatric guppies showed similar responses to all of the models (F2,21 = 1.2, p = 0.3). Results of G-tests comparing the numbers of guppies that pecked or did not peck the prawn model head zone (see electronic supplementary material, table S4) showed a similar pattern. Finally, a survival analysis was conducted to estimate hypothetical survival times for guppies that entered the prawn head zone (figure 2). Allopatric guppies were 2.8 times more likely to be ‘caught’ than sympatric guppies (hazard ratio: 2.8, p < 0.0001). A separate analysis on all guppies, which took into account the different spot colours of the models, was also conducted; guppies exposed to the orange-spotted prawn were 60 per cent more likely to be ‘caught’ than those exposed to the unspotted model (hazard ratio: 0.414, p < 0.003) and 40 per cent more likely to be ‘caught’ than the green-spotted model (hazard ratio: 0.583, p < 0.0001). This survival analysis was conducted assuming that a prawn would catch a guppy that spent three or more seconds in the prawn head zone for 3 s; relaxing this assumption, and instead assuming one second, produced qualitatively similar results (not shown).

(b) Experiment 2: live, novel crayfish
To determine if orange spots on an unfamiliar predator can act as a lure, we exposed guppies from all four populations to crayfish with pincers painted with orange or brown (control) spots. We broke the dataset into four ‘treatments’: sympatric guppies with orange or brown spotted crayfish, and allopatric guppies with orange or brown spotted crayfish. In a preliminary analysis that included all three crayfish zones (head, middle and tail), the interaction between the zone and the treatment was significant and the response to the middle region tended to fall between those to the head and tail zones (data not shown). To simplify the comparison of the results of this experiment with the first experiment (on model prawns), in subsequent analyses we only examined the head and tail regions of the crayfish. We considered several variables as potential covariates; to do this we asked whether the responses of the guppies depended on the identity of the specific crayfish with which they were tested or on the activity level of the crayfish (scored 1–5; see the electronic supplementary material); neither variable was significant and they are not considered further (results not shown). In the initial analysis, there was a significant zone × treatment (population–colour) interaction for all guppies considered together (repeated-measures ANOVA: F3,95 = 6.6, p < 0.0004; electronic supplementary material, table S5), with allopatric guppies spending more time in the head zone than sympatric guppies. To examine this interaction in more detail, separate analyses were performed by population type. Allopatric guppies spent significantly more time in the head zone when pincers were orange-spotted (zone × colour interaction: F1,41 = 5.27, p < 0.03; figure 3) but this interaction was not significant for sympatric guppies (F1,54 = 1.26, p = 0.27; figure 3).

4. DISCUSSION
In summary, our results suggest that orange spots on the pincers of the Trinidadian, diurnal prawn (M. crenulatum) could attract female Trinidadian guppies (P. reticulata) and could thus act as a sensory lure. Several lines of evidence support this hypothesis. First, all guppies were more likely to closely approach the head region of a model prawn with orange-spotted pincers than models with green-spotted or unspotted pincers. As we predicted, this effect of the orange spots as lures was especially striking for sympatric guppies; these guppies tended to avoid the head of the model with unspotted pincers, indicating that they recognized it as a potential predator, until orange spots were added. In the tests with live, non-Trinidadian crayfish, orange spots on the pincers again lured allopatric guppies to the head region; in contrast, guppies that are sympatric with the diurnal prawn were not enticed to the head region of the crayfish when their pincers were adorned with orange spots.

Before testing the sensory lure hypothesis, we first asked whether guppies from populations that co-occur with M. crenulatum recognized our model of this species as a predator by testing them with prawn models with unspotted pincers. As in a previous study with live prawns [30], guppies from populations that co-occur with M. crenulatum were cautious, avoiding the head area of the model prawn, and they spent more time inspecting the tail region; in contrast, allopatric guppies...
Figure 3. Means of the ranked durations that guppies from allopatric and sympatric populations spent in the head and tail zones of the crayfish with orange or brown spots added to its pincers. For each zone, all guppies in this experiment were ranked from lowest to highest duration spent in that zone and the mean rank was calculated for each treatment type, e.g. allopatric brown. There was a significant zone × population interaction for sympatic and allopatric guppies considered together (repeated-measures ANOVA: $F_{1, 95} = 13.79, p < 0.0003$), with allopatric guppies spending more time in the head zone than sympatic guppies. Allopatric guppies spent significantly more time in the head zone when pincers were orange-spotted (zone × colour interaction: $F_{1, 41} = 5.27, p < 0.03$) but this interaction was not significant for sympatic guppies ($F_{1, 54} = 1.26, p = 0.27$). Allopatric populations are denoted by solid lines; sympatric with dashed lines. The colour of each line indicates the colour of the spot on the pincers.

spent more time in the head zone of the model prawn, again in keeping with the results of previous work using live prawns [30]. Next, we used three metrics (duration near, first approaches to and pecks of the models) to ask the question: were guppies lured by orange spots on the pincers; that is, were they more likely to interact with the head region of the model prawn when it was orange spotted? For sympatic guppies, all three metrics indicated that orange spots on the model’s pincers act as lures. The same was true for allopatric guppies although there was not a significant effect of colour for first approaches or pecks, presumably because they are less fearful of the head zone in general. Since there is evidence that M. crenulatum does consume guppies where they naturally co-occur, our results therefore suggest a possible adaptive value for the orange pincer spots to M. crenulatum.

Interestingly, when orange spots were added to the pincers of a live, brown-coloured crayfish (a species unfamiliar to both guppy population types), allopatric, but not sympatic, guppies were apparently lured by the orange spots. For sympatic guppies, this result is not consistent with the sensory lure hypothesis but it is possible that guppies that co-occur with M. crenulatum have evolved to express more caution towards orange spots under situations that are perceived to be especially dangerous or novel (e.g. to show heightened anti-predator behaviour) or to be more aware of and cautious around crustacean predators in general; indeed, the crayfish used in this study were observed pursuing guppies around the test arena. It was not possible to use the same paint on the live crayfish as on the models and this could have been another factor in the differences in the responsiveness by the sympatic guppies to the crayfish models and live crayfish. For example, the orange nail polish used on the live crayfish had peak reflectance at a shorter wavelength than the orange paint used on the crayfish models and was somewhat less reflective at longer wavelengths, yet somewhat more reflective at shorter wavelengths (see electronic supplementary material, figures S3 and S4).

The Trinidadian prawn’s pincer spots could be beneficial to the prawn, beyond acting as a lure, if they contribute to camouflage via cryptic or disruptive colouration [37]. This, however, seems unlikely given that orange spots are not a common motif in the prawn’s natural visual background (personal observation), or in the artificial visual background of our experimental aquaria. Disruptive colouration can be discounted given that guppies spent less time near green-spotted models than orange-spotted ones; this difference between orange and green-spotted models would not be observed if the spots were simply acting to disrupt the characteristic outline of the pincers. We can also rule out the possibility that the orange spots were simply more conspicuous than the green spots; guppy-specific measures of brightness and chroma for the two colours of paint used for the prawn model spots suggest that the green paint is slightly brighter than the orange paint and is at least as chromatic (electronic supplementary material; see table 1 in Rodd et al. [23]). While it seems unlikely that the pincer spots serve a role in intraspecific communication (see §1 and [24,26]), additional studies of M. crenulatum sensory ecology are required to fully address this issue. Other animals in the Trinidadian streams are also attracted to orange-coloured objects, including R. hartii (F.H.R. 2005, 2008, personal observation), a small omnivorous fish; quantifying the relative contribution of guppies and other orange-attracted prey to the diet of juvenile and adult M. crenulatum could provide insights into the role sensory biases play in community interactions. Finally, as M. crenulatum is widely distributed throughout the West Indies, Venezuela and Panama and shows considerable variation in colouration [38], it would be interesting to compare pincer colour patches (and the contrast of the patches with the background colour of the chelae) of prawns from Trinidad with prawns from other areas where the prawn has coexisted with guppies historically (e.g. Venezuela) and to areas where guppies have recently been introduced.

There is still much to learn about the potential effects of prawn predation on guppies. Further studies need to be conducted in future to compare the spectral attributes of orange spots on male guppies, on prawn pincers and in the environment (e.g. orange fruit) to resolve how guppies perceive the orange-spotted pincers. If the prawns, with their alluring pincers, exert significant predation pressure on guppies, this sensory lure could be an added component of selection on guppies’ perception and responses to orange. This could lead to a very complicated network of direct and indirect feedbacks that could affect female mate choice preferences and male colouration [4] and contribute to documented inter-population variation in the strength of female mate
preferences and male colour patterns [20–22,24,39]. For example, if females in some populations have evolved resistance to orange prawn spots, this could lead to an increase in the threshold of the preference for orange on males. This situation could be dynamic, such that the prawn spots evolve towards matching the characteristics (e.g. size, shape, hue and saturation) of the spots on male guppies while the males’ spots evolve away from the characteristics of prawn spots. If the predation pressure from prawns is strong enough, females might evolve to base their mate choice preference on male traits other than their orange spots (e.g. spots of other colours, tail size), but that does not seem to be the case for guppies from the three M. crenulatum sites that have already been examined [20]. Alternate evolutionary responses of females could include a heightened ability to separate responsiveness towards the lure from responsiveness towards the original targets of their bias, such as food or mates [4,40]. Selection could also favour enhanced anti-predator behaviours so that females could better elude prawns’ attacks; this could explain the wariness of our sympatric females to the live crayfish. The next logical step to fully explore the utility of these orange spots to the prawns will be to conduct experiments using live M. crenulatum individuals, preferably in the field, to determine the relative importance of guppies in their diet (both as juveniles and as adults), to quantify the intensity of their predation on guppies compared with other predators and to estimate the efficacy of orange spots in increasing their capture rates.

This study suggests a new dimension to the relationship between natural selection and sexual selection acting on Trinidadian guppies, and it extends our understanding of the ways in which sensory biases can be co-opted by predators to hunt their prey. It is possible that predators are exploiting the sensory biases of their prey (whether it be in the context of mating or foraging) more often than we are aware, especially when signals are undetectable by human observers (e.g. high-frequency sound waves, particular pheromones). If predators are able to exploit signals that contribute to mate choice, those female preferences may become costly, and selection could act to alter female mate choice.

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