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RESEARCH ARTICLE

Pheromones exert top-down effects on visual recognition in the jumping spider *Lyssomanes viridis*

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SUMMARY

In diverse and productive habitats, predaceous arthropods are expected to frequently encounter dangerous conspecifics and heterospecifics. This should make quick and accurate discriminations between species and sexes adaptive. By simultaneously sampling both visual cues and pheromones, and by utilizing stringent species- and sex-specific visual recognition templates, an individual should be able to increase both its speed and accuracy in making such discriminations. We tested for the use and stringency of visual recognition templates in the jumping spider *Lyssomanes viridis* by presenting males with animated images of conspecifics, heterospecifics and composite images that combined the facial coloration and morphology of one sex or species with the leg coloration of another. Males' courtship *versus* threat displays indicated whether a stimulus was perceived as a potential mate or a threat. By comparing males' visual inspection times of, and display types towards, the various images in the presence *versus* absence of female pheromones, we were able to deduce whether males tend to inspect a subset of the color pattern and morphological features that make up their conspecific recognition templates (i.e. those on just the face or just the legs), or all features, and whether this changes in the presence of pheromones. We found that the male recognition template for conspecific female was surprisingly coarse, whereas the recognition template for conspecific male, and especially the male face, was more specific. Pheromones hastened the recognition of images with coloration and morphology closely matching those of conspecifics, presumably by activating conspecific visual recognition templates. When males were presented with an image that was, overall, a poor match to a conspecific female, but that contained a subset of female or female-like features, female pheromones usually did not hasten recognition, but did increase the likelihood that the image would be identified as a female. Taken together, our data suggest that males examined features on both the face and the legs in both the presence and absence of pheromones, and that female pheromones tipped the balance in favor of a female identification when a male was unsure how to categorize an incongruous set of visual features.

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Key words: vision, chemoreception, top-down processing, attention, multimodal, crossmodal interactions.

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INTRODUCTION

Productive habitats, such as forests, grasslands and coral reefs, abound with a diversity of arthropods, many with overlapping microhabitat ranges (Ødegaard, 2000; Hochkirch and Gröning, 2012; Plaisance et al., 2011). The sensory and cognitive task of locating and correctly identifying relevant individuals, such as potential mates and rivals, is not a trivial one, especially when similar species inhabit the same microhabitats at the same times of year (Gröning and Hochkirch, 2008). Although many arthropods are known to use chemical cues to locate relevant individuals in their environments (Nation, 2002), airborne and waterborne chemical cues can be difficult or even impossible to localize in certain conditions, for example on a breezy day or in turbulent waters (Willemse and Takken, 1994; Murlis et al., 2000; Weissburg and Zimmer-Faust, 1993; Weissburg and Zimmer-Faust, 1994), and contact chemical cues can only be detected if an individual happens to cross a path recently traversed by a conspecific (Cook, 1985; Baruffaldi et al., 2010). Furthermore, among taxa that are potentially dangerous to one another, such as jumping spiders, accurate identifications can mean the difference between life and death (Jackson and Pollard, 1997; Harland et al., 2012). Thus, to increase the probability and accuracy of detection and discrimination, selection should favor recognition of species- and sex-specific cues in additional modalities, such as vision (Johnstone, 1996; Endler, 2000; McLennan, 2003).

Besides serving as redundant indicators of species and sex, visual and chemical cues may interact *via* a combination of bottom-up and top-down cognitive processing. Bottom-up processing refers to the raw data inputted to the brain by sensory receptors, whereas top-down processing refers to knowledge and expectations that modify an individual's attention to, and interpretation of, the information provided by sensory receptors (Goldstein, 2002). Historically, research into the interactions between bottom-up and top-down processing has focused on unimodal stimuli, but recent work has expanded to include cross-modal interactions between vision, hearing and touch in primates (Spence and Driver, 2004) and between vision and chemoreception in the jumping spider *Evarcha culicivora* (Cross and Jackson, 2009a). In the case of the jumping spider, the odor of a mosquito (its preferred prey) was found to increase the likelihood that *E. culicivora* would visually locate a dead mount of a mosquito, presumably by activating a visual search template (i.e. mental representation of an object), which enabled it to locate the mosquito faster (Cross and Jackson, 2009a). The initial

detection of mosquito odor would be considered bottom-up processing, whereas the activation of the visual search template would be considered top-down processing. If a similar interaction between chemical and visual cues were to operate in the context of conspecific recognition, multimodal cues could increase not only the probability and accuracy, but also the speed of conspecific detection and discrimination.

An individual could further boost its visual recognition speed through the use of multiple high-stringency visual recognition templates, not for entire animals, but for isolated color pattern or morphological features, such as those on the face or legs. In theory, this could allow an individual to make accurate identifications without having to inspect entire animals. The adaptive benefit of isolated-feature templates would seem to be pronounced in jumping spiders, because the principal eyes, which are specialized for the perception of shape and pattern, have an unusually small field of high-acuity vision, i.e. a 'fovea', subtending a solid angle of approximately 0.6 deg (Williams and McIntyre, 1980). The retina extends dorsally and ventrally beyond the 0.6 deg fovea, encompassing a roughly 21 deg vertical field of low-acuity vision. The horizontal visual field extends only slightly beyond the 0.6 deg fovea in the dorsal and ventral regions of the retina, resulting in an oblong boomerang-shaped retina (Williams and McIntyre, 1980; Land, 1969a). These eyes appear to at least partially compensate for their narrow visual field by scanning over a horizontal range of 45–50 deg (which is a much broader range of motion than that observed in other spiders), as well as an unmeasured but observed vertical range (Land, 1969b; Land, 1985). However, scanning may take some time, retarding recognition time and, in turn, reaction time. Stringent visual recognition templates for isolated features should enable accurate identifications on their own, in the absence of pheromones. However, an individual could further sharpen its accuracy, or alternatively, get by with imperfect recognition templates, by attending to chemical cues when available.

It is well established that female salticids secrete contact pheromones in dragline silk, as well as airborne pheromones (Jackson, 1987; Clark and Jackson, 1995; Taylor, 1998; Willey and Jackson, 1993; Cross and Jackson, 2009b; Jackson and Cross, 2011; Nelson et al., 2012). In some species, female pheromones have been found to escalate male–male conflict (Cross et al., 2007; Cross and Jackson, 2009c). There is also good evidence that both male and female color patterns and morphology (which are sexually dimorphic) in many cases function as species- or sex-specific signals (Crane, 1949; Cross et al., 2006; Lim and Li, 2006; Lim et al., 2007; Lim et al., 2008; Li et al., 2008; Nelson, 2010). Nearly all male salticids, including *Lyssomanes viridis*, the species used in the present study, will not initiate visual displays without first making visual contact with another spider. Pheromones are not a sufficient releaser (see Question 1, Results) (Jackson, 1992; but see Taylor and Jackson, 1999). With the exception of the mosquito study (Cross and Jackson, 2009a) described above, bottom-up and top-down interactions between chemical and visual stimuli have not been explored.

The mosquito study (Cross and Jackson, 2009a) raises an important point about past studies that have been designed to isolate visual cues from chemical cues. Although contact chemical cues are usually rigorously excluded from such experiments, airborne chemical cues are not. However, airborne pheromones are likely to be present in any laboratory that houses the species being researched, especially if the experimental testing and animal housing rooms are in close proximity or share the same heating, ventilation and cooling (HVAC) system. Indeed, airborne pheromones may have increased the probability of visual detection and recognition in past studies. To address this, we set up a cleanroom, which we used to exclude chemical cues from experiments designed to evaluate the effect of visual cues alone. This enabled us to disentangle the independent and interacting roles of visual and chemical cues in conspecific recognition. Specifically, we tested the following questions: (1) are visual cues alone sufficient to elicit courtship and agonistic behavior; (2) do female pheromones hasten the visual identification of conspecifics (presumably by activating visual recognition templates); (3) how stringent are males' visual recognition templates for conspecific male and female features; and (4) do males examine a subset of the features that make up their conspecific recognition templates (i.e. those on just the face or just the legs), or all features, and does the number of features examined decrease in the presence of female pheromones?

To test our predictions, we presented male *L. viridis* jumping spiders (Salticidae) with a variety of animated images, including conspecifics, syntopic heterospecifics and composite images that combined the facial (including pedipalpal and cheliceral) coloration and morphology of one sex or species with the leg coloration of another. (Syntopy, in this study, was defined as the co-occurrence of adults of different species on the same tree branches at the same times of year.) A black circle served as a negative control. Animated images were presented in the absence *versus* presence of female pheromones, which we expected to activate conspecific search templates and hasten recognition. We observed how long males spent looking at each image prior to displaying, and whether they threatened, courted or did nothing in response. We expected that males would threaten images they perceived as conspecific males, court images they perceived as conspecific females and avoid (or, as is more easily quantified, ignore) images they perceived as heterospecifics. Each image was designed to test one or more of the four questions above, as is delineated in Table 1.

The types of comparisons necessary to answer Questions 1 and 2 are intuitive, but those for Questions 3 and 4 are more complex, so we will go through them here. To evaluate Question 3, i.e. the stringency of recognition templates, we judged images that elicited responses from fewer males, or that elicited a mixture of courtship and threat responses, to be poor matches to conspecific visual recognition templates. In addition, images that elicited quicker responses (i.e. shorter inspection times) in the presence of conspecific female pheromones than in their absence were judged to be closer matches to males' conspecific visual recognition templates than images that elicited similar (and relatively slow) reaction times in the presence *versus* absence of pheromones. This judgment was based on evidence from the mosquito study (described above) that chemical cues prime search images, as well as unimodal studies suggesting the use of search images by salticids (Cross and Jackson, 2010; Jackson and Li, 2004).

To evaluate Question 4, one's first inclination might be to show males images of an isolated face or a set of disembodied legs to determine whether the detection of either the face or the legs is sufficient to elicit an adaptive display. If males were to display at these isolated body parts, it would indicate that males need only inspect either the face or the legs to make identifications. However, it would not tell us whether this is what males actually do when presented with an intact spider. In contrast, if males were unresponsive to isolated body parts, we would be unable to conclude the converse, that males must inspect the coloration and fine morphology of entire spiders in order to respond to them appropriately. An alternative explanation for this result could be that males make one or a few broad sweeps over an object with the

Table 1. Images presented to males

Image presented	Description Template specificity question (Question 3)	Questions tested
	**Control male *Lyssomanes viridis*** (♂♂)	**1,2**
	**Control female *L. viridis*** (♀♀)	**1,2**
	***L. viridis* female face without red spot + male legs** (♀♂) Is the male visual recognition template for conspecific female or male confined to features on just the face or just the legs? If so, which?	**3,4**
	***L. viridis* male face + unstriped legs** (♂♀) Is the male visual recognition template for conspecific female or male confined to features on just the face or just the legs? If so, which?	**3,4**
	**Male *L. viridis* face + blackened legs** (♂**B**) Do males look specifically for striped legs, or just dark legs?	**3**
	***Hentzia palmarum* face + male *L. viridis* legs (H♂)** Do males look for specific features on *L. viridis'* face, or simply for dark coloration?	**3**
	***H. palmarum* face + blackened legs (HB)** Is a spider with *L. viridis*-shaped legs, *L. viridis*-like leg-to-body proportions, and predominately dark coloration recognized as a conspecific male?	**3**
	***H. palmarum*** Do males distinguish between unaltered conspecific and heterospecific males with dark coloration on both the legs and face?	**3**
	**Black circle** Is the outline of a spider required to elicit a threat response, or will any dark-colored spider-sized object elicit a threat?	**3**
	***L. viridis* female without red forehead spot** (♀–) Does the red spot facilitate recognition of conspecific females?	**3**
	***Thiodina sylvana*** Does overall light coloration match the conspecific female recognition template well enough to elicit courtship?	**3**

principal eyes to determine its rough outline, in order to classify it broadly as a 'spider' or 'salticid,' and then re-scan a few specific regions more carefully in order to gather fine details of the object's color pattern and morphology. The anterolateral eyes, which face forward and have a broad visual field of relatively coarse resolution (Land, 1969a; Blest, 1983), may also play a role in defining gross shapes and boundaries. Our preliminary work showed that males would not respond to isolated body parts unless they had been primed by intact images of spiders, and Drees (Drees, 1952) showed that males were more likely to display at two-dimensional model spiders with three pairs of legs than those with one pair. This suggests that males must detect the outline of an entire spider to recognize it as such, but does not indicate whether males are inspecting the color pattern and fine morphology of entire spiders, or of the face or the legs only, to make species and sex discriminations. We therefore took a different approach to Question 4. We first looked at whether, when pheromones were absent, the majority of males agreed on the identity of entire images of spiders with features only partially matching those of the conspecific male or female. If males were to agree on the identity of most images, especially the male–female hybrid images (Table 1, rows 3 and 4), then we would be able to conclude that males inspect features on both the face and the legs. To then determine whether males inspect features on just the face or just the legs when pheromones are present, we tested whether

males spent less time examining the control images and the male–female hybrid images before displaying at them when pheromones were present than when they were absent, and if so, whether males became divided as to the identity of the hybrid images when pheromones were present. If males were to spend less time examining the controls and the male–female hybrid images in the presence of pheromones than in their absence, and as a result, were less likely to agree on the identity of the male–female hybrid images in the presence of pheromones, it would suggest that they inspect features on either the legs or the face, but not both, when pheromones are present (Table 2, row 1). Alternatively, if males were to examine control images, but not male–female hybrid images, for less time in the presence of pheromones, and agreed on the identity of male–female hybrid images when pheromones were absent, and either agreed or disagreed as to the identity male–female hybrid images when pheromones were present, it would suggest that males examine features on both the face and the legs in both the presence and absence of pheromones (Table 2, row 2). In this latter scenario, if males disagreed on the identity of the male–female hybrid images, it would likely be because different males gave different weights to the chemical and visual features of a stimulus. Although an alternative explanation for the former scenario (Table 2, row 1) could be that males examine features on both the face and the legs in both the presence and absence of pheromones, but that they examine features more quickly when pheromones are present, we think this explanation is unlikely because an examination of incongruous features is expected to cause confusion and retard recognition relative to the controls.

We used the jumping spider *L. viridis* as our study species. *Lyssomanes viridis* is a translucent green, leaf-dwelling forest species endemic to the southeastern United States. The legs and abdomen of a mature male are covered with black stripes (Table 1, first image). Males also have red hairs on the dorsal and dorsofrontal region of the cephalothorax, and exaggerated reddish-brown chelicerae, which scale positively allometrically with body size (Tedore and Johnsen, 2012). Mature females, in contrast, have a crown of red hairs against a background of white hairs on the dorsal and dorsofrontal regions of the cephalothorax (Table 1, second image). We have observed numerous salticid species, with visual features partially matching those of *L. viridis*, on the same trees at the same times of the year as *L. viridis*, including *Thiodina sylvana*, *T. puerpera*, *Hentzia palmarum*, *H. mitrata* and *Phidippus otiosus*. Thus, to avoid predation, there would seem to be an adaptive value to high-fidelity conspecific recognition templates in *L. viridis*.

MATERIALS AND METHODS

Subjects and housing

Immature *Lyssomanes viridis* (Walckenaer 1837) were collected by beating American holly trees (*Ilex opaca*) along the Black Creek Greenway (35°49.3′N, 78°47.1′W) and in William B. Umstead State Park (35°51.9′N, 78°45.2′W) in Wake County, NC, USA, in late March 2010 and early April 2011. Over the course of 3 years and the collection of 979 juvenile *L. viridis* at this time of year, we have never found a mature individual. Adults die by late summer (Richman and Whitcomb, 1981; C.T., unpublished), so juveniles should not have had the opportunity to view the adult phenotype since the previous summer, if at all. *Lyssomanes viridis* is non-social, paternal care is non-existent, and the mother leaves the nest soon after her young gain the use of their principal eyes; thus juveniles may have never seen the adult phenotype (for elaboration, see Discussion, Limitations of this study). All spiders had molted to sexual maturity prior to being run in experimental trials.

Spiders were individually housed in 10×10×10 cm clear plastic boxes. Subjects were visually isolated from one another by white paperboard barriers and were illuminated from above by two full-spectrum (including ultraviolet) fluorescent mercury vapor tubes (T8, 32 W, 48 inch, Duro-Test Lighting's Vita-Brite, Philadelphia, PA, USA). The room temperature was held constant at 24°C. Spiders were given simulated leaves, in the form of an 8×10 cm piece of green paper on top of each box, and a folded 6×14 cm piece inside each box. Upon maturation, the folded piece was removed from female boxes to encourage silk deposition on the lid (see Trials including chemical cues, below). The light cycle was regulated by a digital timer, which was programmed to turn on at dawn and off at dusk in their natural habitat, accounting for seasonal changes in day length. During the weeks leading up to experiments, spiders were fed eight *Drosophila* and were misted with filtered water two times per week. Four days before experimental trials began, we increased feeding and misting frequency; spiders then received four *Drosophila* and a light misting daily, and this continued until all behavioral trials were complete.

Experimental arena and video presentation

Arenas were 10×10×5 cm clear plastic boxes whose floor and walls were surrounded by white paperboard. The arena's ceiling was covered by white translucent vellum to diffuse the room's ambient lighting. A webcam (Logitech QuickCam Pro for Notebooks, Logitech, Newark, CA, USA), pointed through a small opening in one of the paperboard walls, was used to monitor the spider's behavior. When placed in the arena, males typically stood still for several seconds before beginning to move about and explore the arena. A male was considered habituated to the arena when he began moving about. At this point, one of the paperboard walls was removed and a high pixel density (10.6 pixels mm^{-1}) computer screen (Fujitsu Lifebook U820, Tokyo, Japan) was slid into view, which displayed a life-size animated image of a spider (described below).

Visual stimuli

Table 1 shows the various images used and the hypotheses they were designed to test. Images were generated from photographs taken by

Table 2. Question 4 – alternative experimental outcomes and their interpretations

Time spent examining control male and female	Time spent examining male–female hybrids	Agree/disagree on identity of male–female hybrids when pheromones are…		Interpretation
		Absent	Present	
Pheromones present < Pheromones absent	Pheromones present < Pheromones absent	Agree	Disagree	Males examine fewer visual features in the presence of pheromones than in their absence. (They examine features on either the face or the legs, but not both.)
Pheromones present < Pheromones absent	Pheromones present = Pheromones absent	Agree	Agree or disagree	Males examine features on both the face and the legs in both the presence and absence of pheromones.

C.T. (*L. viridis*) and J. Barnes (*H. palmarum* and *T. sylvana*). Various alterations were made to the original photographs in order to standardize the background, size and symmetry of the experimental stimuli. All manipulations were performed using Photoshop CS4 (Adobe, San Jose, CA, USA).

The background was removed from all photographs and replaced with a uniform white. The leg arrangements were made symmetric by mirroring the legs from one side of the body to the other. For the female *L. viridis*, we controlled for incidental differences in leg positioning and posture between males and females by combining a female face with male legs and coloring the legs a uniform, unstriped, 'female' green. We scaled the male and female *L. viridis* cephalothoraxes, as well as the *H. palmarum* cephalothorax, so that their eyes were the same size across images. The size of a typical *H. palmarum* was accurately reproduced by this method. Because of substantial differences in body proportions, the female *T. sylvana* would have been unnaturally tiny if we had scaled the eyes in the same way, so we instead scaled her entire body (face and legs) to be the same width as the control male and female *L. viridis*. This made her somewhat larger than a real *T. sylvana*; the width of her cephalothorax measured approximately 50% wider than that of two individuals we caught and measured later. The black circle had the same overall area as the male *L. viridis* image (♂♂).

Because *L. viridis* seem to reflexively orient towards moving stimuli, and to rarely notice motionless conspecifics and potential prey items, entire still images were animated to alternately move to the left or right 3.5 mm with a period of 15 s between movements. Animations were constructed using Adobe After Effects CS4.

Chemical stimuli

Trials including chemical cues

Female *L. viridis* spend most of their time perching upside down on the undersides of leaves or, in the laboratory, on the undersides of their enclosure lids. Here they lay down a flat sheet of silk (i.e. 'nest') to which they cling. For trials including female silk, each male was randomly assigned to a different female's enclosure lid. At the beginning of a trial, the assigned female was temporarily moved from her home lid to a temporary lid. The male was then allowed to climb from his home lid onto the female's silk-covered home lid. The female's 10×10 cm silk-covered lid, with the male perched on it, was used to enclose the experimental arena, which was a 10×10×5 cm clear plastic box. At the end of each trial, the female's home lid was returned to her home enclosure, and the male was returned to his home enclosure. Each female's home lid was re-used by the same male on all of the following days of a given experiment. A male was never given a female lid on which another male had previously perched.

Trials excluding chemical cues

To counteract any lasting effects of chemical cues, all conspecific chemical cues, including both silk and airborne cues, were excluded 24 h a day throughout the duration of the 10 day experiment that excluded chemical cues. This was accomplished by making all transfers between home enclosures and experimental arenas in a cleanroom and by completely sealing off home enclosures and arenas from air potentially contaminated by conspecific chemical cues. The cleanroom was set up in a vacant classroom located 34 m down the hall from the spider housing and experimental testing room. The classroom had an independent HVAC system, separate from the rest of the building. All of the windows were opened and the room's HVAC fans were turned on high. (The HVAC intake vent and windows looked out on a grassy internal courtyard, which is not *L. viridis* habitat.) This created positive air pressure in the room relative to the hallway, which was verified by observing the direction of airflow at the entrance to the cleanroom. This setup prevented air from the spider housing and experimental testing room (which did not adjoin the courtyard) from wafting into the cleanroom.

The day before a series of chemical cue exclusion experiments began, home enclosures were soaked in 1% bleach [sodium hypochlorite (NaClO) and sodium hydroxide (NaOH)] for 30 min, scrubbed with a sponge and rinsed with water. Enclosures were then scrubbed with 70% ethanol, using a neoprene-gloved hand, and allowed to air dry. Each spider was taken, one at a time, into the cleanroom and placed into one of these boxes, enclosed by a friction-fitting lid. The box itself was enclosed in a 3.8 l resealable plastic bag as a secondary barrier against chemical cues. Between transfers, the experimenter (C.T.) scrubbed her hands with an alcohol-based hand sanitizer that was fragrance- and dye-free (Dial Co. hand sanitizer, Scottsdale, AZ, USA), and then rinsed her hands with tap water. Each boxed and bagged spider was then taken back to the spider housing and experimental testing room.

Each spider was assigned its own experimental arena, which had been cleaned using the same protocol that was used for home enclosures above. Each arena was stored in the cleanroom in its own 3.8 l resealable bag when not in use. Before each experimental trial, a spider was taken into the cleanroom and transferred from its home enclosure to its assigned experimental arena. The experimental arena had a friction-fitting lid; however, as a secondary barrier against chemical cues, the seam between the lid and box was sealed using 1.3 cm transparent Scotch tape. The spider, in its arena, was then taken back to the spider housing and experimental testing room for its trial. After completion of its trial, the spider, in its arena, was carried back to the cleanroom, transferred back to its home enclosure, and brought back to the spider housing and experimental testing room. Between trials, the experimenter scrubbed her hands with hand sanitizer and rinsed with water as described above. To prevent the build-up of silk in experimental arenas over the course of an experiment, each arena was wiped clean with a fresh paper towel daily.

Experimental procedure and sequence of experiments

Once a video was slid into view, a spider was given three chances to orient towards the screen and to examine and respond to the image. If a spider oriented in such a way that it was standing at an angle greater than or equal to 90 deg relative to the plane the spider image was 'standing' on, it was allowed to orient towards and examine the image once again before the trial ended.

For the first round of chemical cue inclusion experiments, we presented 32 naïve males with these images: ♂♂, ♀♀, ♀♂, ♂♀, ♂B, H♂, ♀– and *T. sylvana* (Table 1). Each individual saw these images in a different random order, one image per day, on eight consecutive days. Three weeks later, these same males (minus four that had died) were presented with four additional images, ♂♂, HB, *H. palmarum* and black circle (Table 1), again presented in random order, one image per day, on four consecutive days.

We conducted the chemical cue exclusion experiments the following year with a new set of 32 males who were presented with all of the images used in the previous year except for the black circle. Each individual saw these images in a different random order, one image per day, on 10 consecutive days. Five images (♀♂, ♂♀, HB, *H. palmarum* and *T. sylvana*) elicited significantly different responses between chemical cue inclusion and exclusion treatments. To ensure that these different responses were not due to an unknown condition being different across years and subjects, we re-ran the

males from the chemical cue exclusion experiment in these five treatments, this time in the presence of female silk. As before, images were presented in random order, one image per day, on five consecutive days.

Behavioral and statistical analyses

Lyssomanes viridis' stereotypic courtship displays are characterized by an extension of the forelegs and alternate rapid flicking of the fore metatarsi with the pedipalps extended straight above the head (Richman, 1982). Stereotypic threat displays are also characterized by raised forelegs, but the legs, rather than being flicked, simply alternate between being fully extended and partially bent at the joint connecting the femur to the patella, and the pedipalps are lowered in a neutral position (Tedore and Johnsen, 2012). For the purposes of this experiment, a display was classified as a courtship if at least one pedipalp and both forelegs were extended above the head, and as a threat if one or both forelegs were raised and held immobile above the head for at least 3 s (Fig. 1).

The time males spent looking at each image was scored as the time between the male's reflexive orientation towards the movement of an animation and his resulting visual display (either threat or courtship). If a male oriented multiple times to an image without displaying, with long bouts of exploration in between, we only counted the final orientation. There were two justifications for beginning timing from the final orientation. The first was that numerous males spent inordinately long periods of time examining an image when oriented perpendicular or upside down relative to the image. Drees' (Drees, 1952) work showed that the male salticid *Epiblemum scenicum* is less likely to recognize and display at spider images when not oriented in the same plane as the image, which corroborates this observation. The second justification was that in preliminary trials, when males were allowed to orient and display multiple times towards a non-control image, they would often give opposite displays on successive orients. This suggests that the same male can evaluate the same set of stimuli differently at different time points, and that the same image may frequently have been perceived as a new individual each time the spider oriented towards it. Individuals who did not display at a given animation were excluded from the inspection time analyses involving that particular image. Because of the wide variability in visual inspection times, and thus the relatively weak power of our between-group comparisons, we also analyzed data from a within-group preliminary experiment. In this experiment, a naïve group of males was shown the control male and female images in the presence *versus* absence of female pheromones, in random order, one presentation per day, on consecutive days. Due to the preliminary nature of this experiment, airborne chemical cues were not specifically eliminated in the chemical cue exclusion treatments of this experiment.

All within-group comparisons of males' threat *versus* courtship responses to the various images were made using McNemar's test of correlated proportions; between-group comparisons were made using Fisher's 2×2 exact test. Between-group comparisons of visual inspection times were made using the Mann–Whitney *U*-test; within-group comparisons were made using the Wilcoxon signed-ranks test. All reported *P*-values are two-tailed.

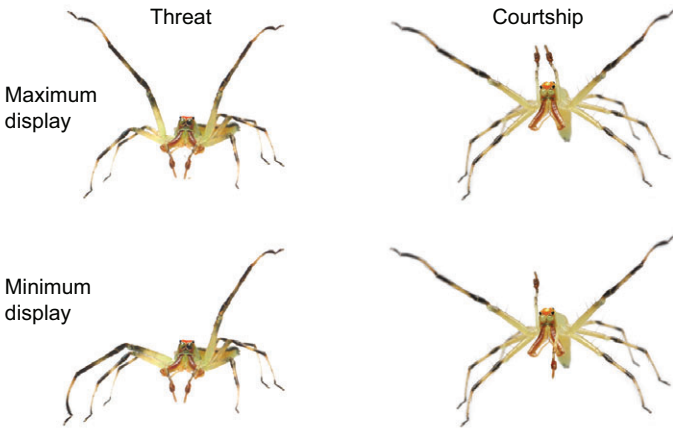

Fig. 1. Threat and courtship stances of *Lyssomanes viridis*. A display was scored as a threat if one ('minimum') or both ('maximum') of the forelegs were raised and held immobile for at least 3 s. A display was classified as courtship if both forelegs and one ('minimum') or two ('maximum') pedipalps were raised.

Cautionary note about video recordings

In the chemical cue inclusion experiment, numerous trials were accidentally not video recorded. The display type was noted in these trials, but visual inspection times could not be calculated. Thus, the sample sizes for inspection time, as shown in Figs 2–5, are smaller for this treatment than for the chemical cue exclusion treatment.

RESULTS

Question 1 – are visual cues alone sufficient to elicit courtship and agonistic behavior?

The majority of males threatened the control male image (♂♂) (e.g. supplementary material Movie 1) and courted the control female image (♀♀) (Fig. 2). This did not change across chemical cue treatments for either the control male (Fisher's exact test, P=0.76) or the control female (Fisher's exact test, P=0.13).

We noted that, as expected, in both the presence and absence of chemical cues, no male initiated a visual display without first orienting towards and examining the animated image. Visual displays were always clearly directed toward the animated image.

Question 2 – do female pheromones hasten the visual identification of conspecifics (presumably by activating visual recognition templates)?

Males spent significantly less time examining the control male image, prior to displaying, in the presence of female pheromones than in their absence. This was true for both the within- and between-group comparisons (Wilcoxon signed-ranks test, P=0.024; Mann–Whitney *U*-test, P=0.027; Fig. 2). Males also spent less time examining the control female image in the presence of female pheromones than in their absence. This difference was significant for the within-group comparison, which was the more powerful comparison, but not the between-group comparison (Wilcoxon signed-ranks test, P=0.023; Mann–Whitney *U*-test, P=0.17; Fig. 2).

Question 3 – how stringent are males' visual recognition templates for conspecific male and female features?

Images with predominately dark coloration and male–female hybrid images

In the absence of female pheromones, males threatened all of the images that had more than just a small spot of dark coloration on the legs and/or face as often as they threatened the control male, including the male face with blackened legs (♂B) (McNemar's test, P=1.00), the heterospecific face with male legs (H♂) (McNemar's test, P=0.69), the heterospecific face with blackened legs (HB)

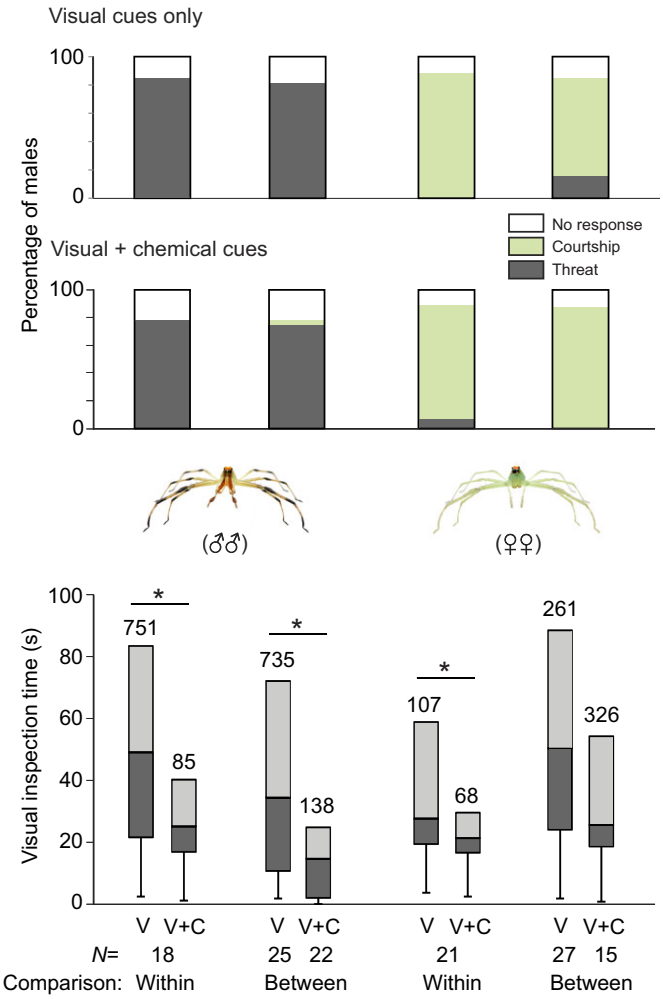

Fig. 2. Male display types (top two bar graphs, within-group *N*=27; between-group *N*=32) and visual inspection times (bottom box plot) in response to the control male and control female images in the absence *versus* presence of conspecific female pheromones. Box plot values consist of the median (center line), upper and lower quartiles (upper and lower edges of box), and maximum and minimum values (whiskers). To keep the vertical dimension of the box plot at a readable scale, upper whiskers were not plotted, but their values are indicated by the number displayed above each box. An * indicates a significant difference in the amount of time males spent inspecting an image in the presence *versus* absence of pheromones. V, visual cues only; V+C, visual + chemical cues; within, within-group comparison; between, between-group comparison.

(McNemar's test, *P*=0.51), *H. palmarum* (McNemar's test, *P*=0.15), the female face with male legs (♀♂) (McNemar's test, *P*=0.75) and the male face with unstriped yellow legs (♂♀) (McNemar's test, *P*=1.00; Figs 3, 4).

When female pheromones were added, males continued to threaten images with predominately dark coloration on both the legs and face. This included the male face with blackened legs (♂B) (Fisher's exact test, *P*=1.00), the heterospecific face with male legs (H♂) (Fisher's exact test, *P*=0.092) and the heterospecific face with blackened legs (HB) (Fisher's exact test, *P*=0.13; Fig. 3). One of these images, however, received significantly fewer displays overall when female pheromones were added: the *H. palmarum* face with blackened legs (HB) (Fisher's exact test, *P*=0.0017). Of these three images, males spent less time examining just one of them in the presence of pheromones: the male face with blackened legs (♂B) (Mann–Whitney *U*-test, *P*=0.022). The remaining images did not differ in the amount of time males spent examining them in the presence *versus* absence of pheromones. This included the heterospecific face with male legs (H♂) (Mann–Whitney *U*-test, *P*=0.82) and the heterospecific face with blackened legs (HB) (Mann–Whitney *U*-test, *P*=0.37; Fig. 3). The black circle, which was only run in the presence of female pheromones, elicited fewer courtships (McNemar's test, *P*<0.0001) and fewer threats (McNemar's test, *P*<0.0001) than the control female and male, respectively.

When female pheromones were added, males became more likely to court images containing light legs and a dark face, or dark legs and a light face. This included the female face with male legs (♀♂) (Fisher's exact test, *P*=0.014), the male face with female legs (♂♀) (Fisher's exact test, *P*=0.0057) and *H. palmarum* (Fisher's exact test, *P*=0.0025; Figs 3, 4). Of these three images, males spent less time, in the presence of pheromones, examining just one of them before displaying at it: the female face combined with male legs (♀♂) (Mann–Whitney *U*-test, *P*=0.0061). The remaining images did not differ in the amount of time males spent examining them in the presence *versus* absence of pheromones. This included the male face with female legs (♂♀) (Mann–Whitney *U*-test, *P*=0.45) and *H. palmarum* (Mann–Whitney *U*-test, *P*=0.42; Figs 3, 4).

Images with predominately light coloration

In the absence of pheromones, males were just as likely to court the female without the red forehead spot (♀–) as they were to court the control female (♀♀) (McNemar's test, *P*=1.00; Fig. 5). Males were split between threatening and courting the light-colored heterospecific, *T. sylvana*, and were significantly less likely to court this image than the control female (McNemar's test, *P*=0.011). When female pheromones were added, males became even more likely to court the female without the red forehead spot (Fisher's exact test, *P*=0.043). Males also became more likely to court *T. sylvana* (Fisher's exact test, *P*=0.0026), and became just as likely to court *T. sylvana* as the control female (McNemar's test, *P*=0.070) when female pheromones were present. Males spent less time examining the female without the red forehead spot (♀–) when female pheromones were present than when they were absent (Mann–Whitney *U*-test, *P*=0.0016). Males did not differ in the amount of time they spent examining *T. sylvana* in the presence *versus* absence of pheromones, however (Mann–Whitney *U*-test, *P*=0.35).

Question 4 – do males examine a subset of the features that make up their conspecific recognition templates (i.e. those on just the face or just the legs), or all features, and does the number of features examined decrease in the presence of female pheromones?

When visual cues were the only ones available, males gave consistent responses to all images except *T. sylvana* (see Question 3 above). When female pheromones were added, males became significantly more likely to court images containing light legs and a dark face, or dark legs and a light face, as well as non-control images that were overall light in color. This included the female face with male legs (♀♂), the male face with female legs (♂♀), *H. palmarum* and *T. sylvana* (see Question 3 above for statistics; Figs 3–5). Of these images, males spent significantly less time, in the presence of pheromones, examining just one of these images before displaying at it: the female face combined with male legs

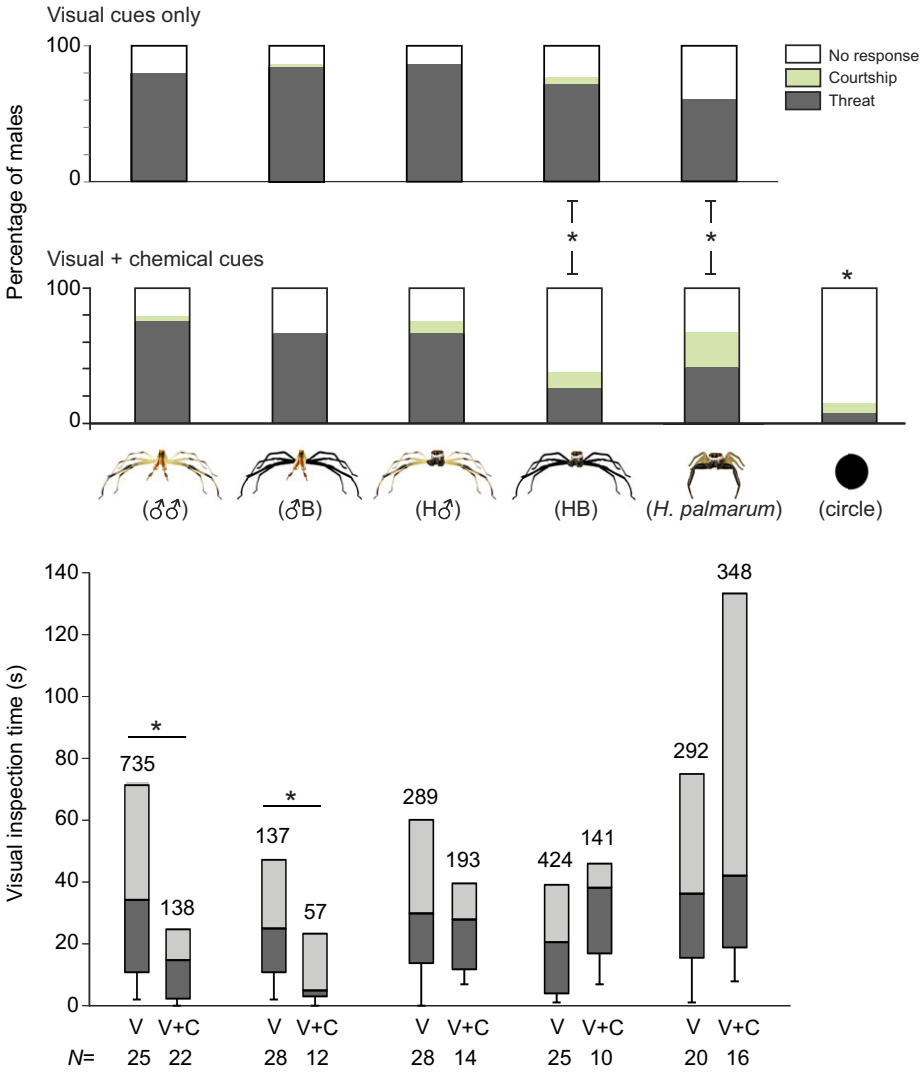

Fig. 3. Male display types (top two bar graphs; top graph *N*=32, bottom graph *N*=32 for the first three images and *N*=27 for the last three images) and visual inspection times (bottom box plot) in response to predominately dark-colored heterospecifics and composite images in the absence *versus* presence of conspecific female pheromones. Box plot values consist of the median (center line), upper and lower quartiles (upper and lower edges of box), and maximum and minimum values (whiskers). To keep the vertical dimension of the box plot at a readable scale, upper whiskers were not plotted, but their values are indicated by the number above each box. In the top two bar graphs, an * spanning the graphs indicates a significant difference in how males responded to the associated image in the presence *versus* absence of pheromones. An * above a single bar indicates a significant difference in how males responded to the associated image compared with the control male. In the box plot, an * indicates a significant difference in the amount of time males spent inspecting the associated image in the presence *versus* absence of pheromones. V, visual cues only; V+C, visual + chemical cues.

(♀♂) (see Question 3 above for statistics). In addition, males were less likely to threaten the heterospecific face combined with blackened legs (HB) than they were to threaten the heterospecific face combined with male legs (H♂) (McNemar's test, P=0.0074) or the male face combined with blackened legs (♂B) (McNemar's test, P=0.0074) (see Discussion for justification of these last two comparisons).

Consistency of responses over time and across subject pools

As noted in the Materials and methods, the 2010 subjects were presented with the control male in the presence of female silk two times, separated by a period of approximately 3 weeks. The subjects' responses to this identical stimulus did not change over this time span (McNemar's test, P=0.73). In addition, subjects from 2010 and 2011 did not differ in their responses to the same combinations of visual and chemical stimuli (Fisher's exact test, ♀♂, P=0.61; ♂♀, P=0.86; HB, P=0.71; *H. palmarum*, P=0.61; *T. sylvana*, P=0.20).

DISCUSSION

Question 1 – are visual cues alone sufficient to elicit courtship and agonistic behavior?

Our results demonstrated that visual cues alone are sufficient to elicit courtship and agonistic displays in *L. viridis* (Fig. 2). By using a cleanroom, this is the first study, to our knowledge, in which all traces of airborne chemical cues were definitively eliminated from behavioral trials designed to isolate visual cues from chemical cues.

Question 2 – do female pheromones hasten the visual identification of conspecifics (presumably by activating visual recognition templates)?

As hypothesized, female pheromones hastened the recognition of conspecific images, as well as a few of the other images, as described below. This adds further support to the idea that salticids are not passive consumers of sensory information, but instead use information from one modality to direct their attention to, and aid perception of, cues in other modalities (Cross and Jackson, 2009a). All tests including pheromones used female pheromones; a follow-up study of the effects of male pheromones (provided they exist) could provide an interesting contrast and bring greater clarity to the results presented here.

Question 3 – how stringent are males' visual recognition templates for conspecific male and female features?

Because males readily threatened most spider-shaped images that were not recognized as female, it was difficult, at first, to assess whether males' recognition templates for male features were more

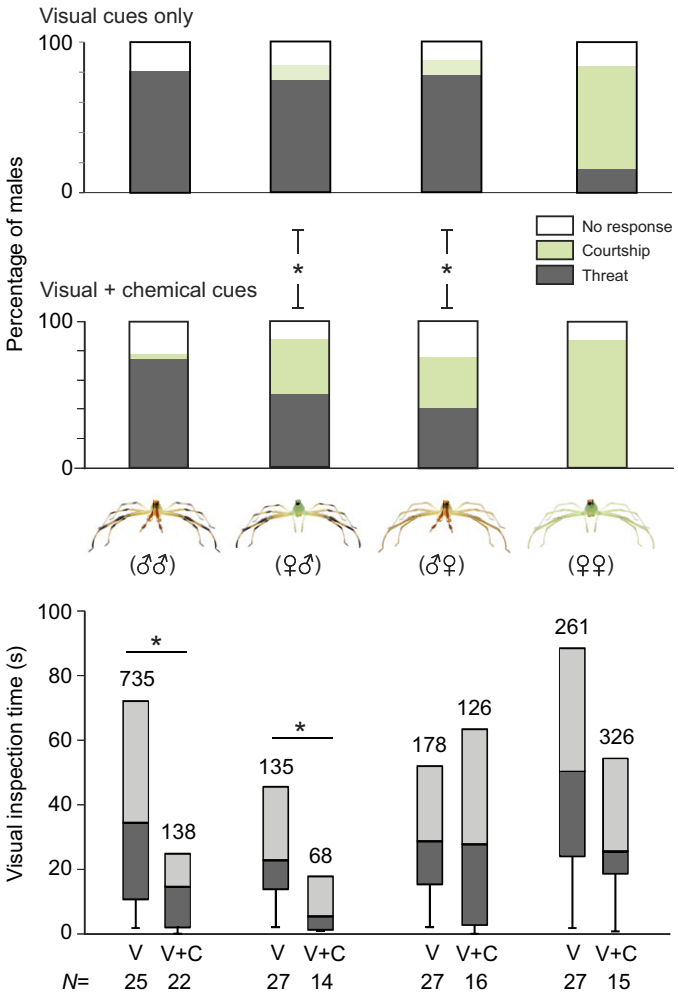

Fig. 4. Male display types (top two bar graphs; *N*=32 for each graph) and visual inspection times (bottom box plot) in response to different combinations of female and male features in the absence *versus* presence of conspecific female pheromones. Box plot values consist of the median (center line), upper and lower quartiles (upper and lower edges of box), and maximum and minimum values (whiskers). To keep the vertical dimension of the box plot at a readable scale, upper whiskers were not plotted, but their values are indicated by the number displayed above each box. In the top two bar graphs, an * spanning the graphs indicates a significant difference in how males responded to the associated image in the presence *versus* absence of pheromones. In the box plot, an * indicates a significant difference in the amount of time males spent inspecting an image in the presence *versus* absence of pheromones. V, visual cues only; V+C, visual + chemical cues.

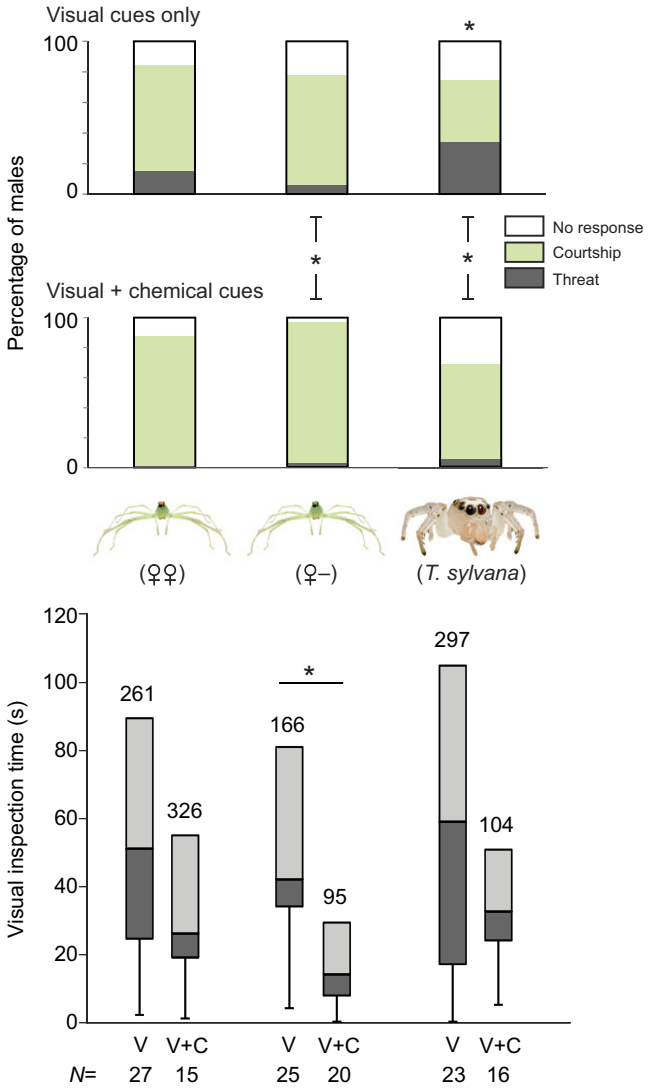

Fig. 5. Male display types (top two bar graphs; *N*=32 for each graph) and visual inspection times (bottom box plot) in response to spider images with predominately light coloration in the absence *versus* presence of conspecific female pheromones. Box plot values consist of the median (center line), upper and lower quartiles (upper and lower edges of box), and maximum and minimum values (whiskers). To keep the vertical dimension of the box plot at a readable scale, upper whiskers were not plotted, but their values are indicated by the number displayed above each box. In the top two bar graphs, an * spanning the graphs indicates a significant difference in how males responded to the associated image in the presence *versus* absence of pheromones. An * above a single bar indicates a significant difference in how males responded to the associated image compared with the control female. In the box plot, an * indicates a significant difference in the amount of time males spent inspecting an image in the presence *versus* absence of pheromones. V, visual cues only; V+C, visual + chemical cues.

specific than a dark spider-shaped object. A study by Nelson and colleagues showed that salticids in the ant-mimicking genus *Myrmarachne* deter attacks from ant-specialist salticids by raising their forelegs when they see another jumping spider (Nelson et al., 2006). Although this was interpreted as a signaling strategy unique to ant mimics, it occurred to us that this could be a more generalized antipredator strategy among salticids. If this were so, it could be difficult to interpret whether a threat display is indicative of conspecific recognition or predator avoidance. However, by comparing the length of time males spent looking at images before displaying at them across chemical cue treatments, we were able to deduce which images were close matches to the conspecific male recognition template, and which were not.

Images with predominately dark coloration and male–female hybrid images

When female pheromones were present, males responded more quickly to (1) the control male (♂♂) and (2) the male face combined with blackened legs (♂B) than when pheromones were absent. This was not true for any of the other images with dark coloration on both the face and legs; rather, males spent just as much time examining these other images in the presence of female pheromones as they had

in their absence. This suggests that males do not make a strong distinction between striped and uniformly black legs, but that specific features of the male face are crucial for conspecific male recognition.

However, a piece of evidence that at first seems to weaken the argument for the specificity of the male facial template is that, when female pheromones were present, males responded faster, often with a threat display, to the image combining a female face with striped male legs (♀♂). If specific features of the male face are an integral part of the recognition template for conspecific male, how could males respond more quickly to this image with a threat? Although speculative, we think this image may have been perceived as a mating pair. During copulation, the male climbs on top of the female, straddling her body with his legs and facing the opposite direction (C.T., unpublished). If one were to view the male from behind, the female's face and male's legs would be the most prominent visual features. When the male and female are just about to begin, or have already begun, copulating, it would behove an intruder male to intercede as quickly as possible. The fact that males responded to this combination of visual features more quickly in the presence of pheromones suggests that males have a recognition template for mating pairs that includes only a subset of the male and female visual features that are necessary for individual recognition. Males may have been divided between threatening and courting this image because of inter-individual variation in strategy upon encountering a mating pair. Some individuals may court in an attempt to lure the female away from her current mate, whereas other individuals may threaten in an attempt to chase away the rival male. Males often threaten and court lone males and females, respectively, who are not facing them (C.T., unpublished), which suggests that males may not be aware of which individual in a mating pair would be most likely to see a visual display.

Conversely, when presented with the 'opposite' image, i.e. one that combined a male face with unstriped yellow legs, males did not respond more quickly to it in the presence of pheromones. Like its 'opposite,' this image could arguably be construed as a mating pair. However, because the male's legs are darkly striped, they should be more prominent than the female's, but instead, are completely absent from the image. If the visual features necessary for the recognition of a mating pair are a subset of those necessary for individual recognition, we would expect features that are prominent from any angle, such as male legs, to be a necessary component of that recognition template. The fact that males did not respond to this image more quickly in the presence of pheromones, but were still split between threatening and courting it, suggests that they had difficulty categorizing this particular combination of features.

One of the more puzzling aspects of our data was the fact that males were less likely to display at the image that combined a *H. palmarum* face with blackened legs (HB) when female pheromones were present than when they were absent. If striped and solid legs are diagnostically equivalent, it is unclear why males should respond differently to this image than they did to the image that combined a *H. palmarum* face with striped legs (H♂). However, if males are simply attending to whether the legs contain black coloration, and pattern is irrelevant, then it is possible that solid black legs were perceived as a more exaggerated, and therefore intimidating, version of spider legs than striped legs, and that males were reluctant to threaten such an intimidating heterospecific. Alternatively, if striped and solid black legs are not diagnostically equivalent, it could be argued that this image contained the fewest color pattern elements in common with conspecific males, and was therefore the least likely to be successfully categorized in the presence of a strong incongruent chemical stimulus. This interpretation implies that, at least in the presence of pheromones, males generally categorized the rest of the images as conspecifics rather than as heterospecifics. While images with congruent conspecific leg and facial features elicited the quickest and most adaptive responses, and thus seemed to match males' recognition templates the best, poorer matches may also have been recognized as conspecifics. However, these identifications would probably take longer to make, due to males being confused by images that were only partial matches to their conspecific recognition templates.

Images with predominately light coloration

Lyssomanes viridis' template for conspecific female appeared to be coarse, i.e. light coloration on the face and legs. The distinct red forehead spot did not facilitate recognition; if anything, males were quicker and more likely to court the female missing the red spot (♀–), although these differences were not significant (Fig. 5). Although numerous males courted *T. sylvana* in the absence of pheromones (e.g. supplementary material Movie 2), significantly fewer males courted her than the control female, which suggests she was perceived as only a poor match to the conspecific female template. To gain further insight into which of these three images most closely matched males' recognition templates, we compared visual inspection times in the presence *versus* absence of female pheromones. Interestingly, both the control female (for the within-group comparison) and the female without the red forehead spot elicited significantly quicker responses in the presence of pheromones, whereas *T. sylvana* did not (Fig. 5). This further supports our above deduction that *T. sylvana* was not as close of a match to the conspecific female recognition template as the other two images. That said, the template for conspecific female is clearly not highly stringent, given that so many males courted *T. sylvana*, even in the absence of pheromones. Perhaps female *L. viridis'* green hue, or slender proportions, as compared with *T. sylvana*'s, are also features (albeit not entirely essential ones) that males cue in on during the recognition decision-making process.

Question 4 – do males examine a subset of the features that make up their conspecific recognition templates (i.e. those on just the face or just the legs), or all features, and does the number of features examined decrease in the presence of female pheromones?

When visual cues were the only ones available, males largely agreed as to the identity of most images, including, most importantly, the male–female hybrid images. This suggests that when pheromones were absent, males examined features on both the face and legs before coming to a decision about the identity of an image. When pheromones were added, males became less likely to agree on the identity of the male–female hybrid images ♂♀ and ♀♂. Males inspected the female face combined with male legs (♀♂) for less time in the presence of pheromones, but inspected the male face combined with female legs (♂♀) for the same amount of time in the presence of pheromones as they did in their absence. Given that males inspected the control male and female images for shorter periods in the presence of pheromones, the ♂♀ result suggests that males were not inspecting fewer features in the presence of pheromones, whereas the ♀♂ result suggests that they were (see Table 2). However, the fact that males were also more likely to court other images with a subset of female-like features (i.e. light legs 2–4 on *H. palmarum* and overall light coloration on *T. sylvana*), but inspected them for the same amount of time in the presence and absence of pheromones, provides somewhat more support for the

conclusion that males inspect features on both the face and legs in both the presence and absence of pheromones.

Another piece of evidence that supports the conclusion that males inspect features on both the face and legs in both the presence and absence of pheromones can be seen in a comparison of males' responses to, and inspection times of, the male face combined with blackened legs (♂B), the heterospecific face combined with male legs (H♂) and the heterospecific face combined with blackened legs (HB) in the presence *versus* absence of pheromones. If, counter to our conclusion above, males were inspecting features on either the legs or the face (but not both), then blackened legs would almost certainly have to have been perceived as diagnostically equivalent to striped legs in order for the inspection time of ♂B to have been significantly lower in the presence of pheromones, especially given our small sample size and concomitant low statistical power to detect a significant difference (N=12 for the chemical cue inclusion treatment). If males perceived blackened legs as diagnostically equivalent to striped legs, then males who examined features on the legs, but not on the face, of HB in the presence of pheromones should have exhibited shorter inspection times than they did in the absence of pheromones, because the legs should have been perceived as congruent with conspecific pheromones, and thus should not have confused males nor delayed their response. This should have drawn down the median inspection time of HB in the presence of pheromones relative to what it was in the absence of pheromones for both HB and H♂. Instead, the median inspection time, as well as the interquartile range, of HB was higher in the presence of pheromones than it was in the absence of pheromones for both HB and H♂ (albeit not significantly so). This is more in line with our tentative conclusion in the previous paragraph that males inspect features on both the legs and face in the presence of pheromones, and that incongruity between leg and facial features, as well as between visual and chemical cues, was confusing to males. More importantly, if, when pheromones were present, males were only inspecting features on the face or legs (but not both), then males who inspected only the face of HB should have responded in the same way that they responded to H♂, by predominately threatening HB. Similarly, males who inspected only the legs of HB should have responded in the same way that they responded to ♂B, again, by predominately threatening HB. Instead, males were significantly less likely to threaten HB than either H♂ or ♂B, which further supports our conclusion that males inspect both the face and legs in the presence of pheromones.

If, indeed, males inspect features on both the face and legs in the presence and absence of pheromones, how can we explain the fact that males inspected the female face combined with male legs (♀♂) for a shorter period of time in the presence of pheromones than in their absence? One explanation could be that when female pheromones are present, males examine the face first, and if the face is a good match to the female pheromones (and is not a male), then they do not bother to examine the legs. However, males who threatened this image in the presence of pheromones tended to inspect the image for less time (although not significantly so) than those that courted it, which argues against this interpretation. Another explanation, as was described for Question 3, could be that this image was perceived as a mating pair, and males have a recognition template for mating pairs that includes only a subset of the male and female features that are necessary for individual recognition. This may facilitate quicker recognition than would be observed in response to other images combining incongruous facial and leg features.

Limitations of this study

Recognition templates may be innate and/or learned. Among non-social arthropods with minimal parental care, such as *L. viridis*, recognition templates for adults are probably mostly innate. In the present study, however, we did not control for the potential effects of learning in the early instars of our study species. Subjects were wild-caught juveniles a few instars away from their terminal molt, during a time when adults are not present in nature (early spring; see Materials and methods). However, during the previous summer, chance encounters with adults, especially with the mother, who guards the nest for approximately 10 days post-hatching (C.T., unpublished), may have occurred. However, a study of the post-embryonic development of the principal eyes of the salticid *Plexippus validus* showed that the principal eyes continue to develop until day 15 after hatching. Eye morphology indicates that the spiderlings are blind during this period. Spiderlings do not begin moving about and do not leave the nest until the eyes have fully developed (Blest, 1988). In *L. viridis*, the mother leaves the nest soon after the spiderlings extend their legs and begin moving about (C.T., unpublished). Thus, if *L. viridis* spiderlings do make visual contact with their mother, it is likely only during the first few days after they gain use of their principal eyes.

Although computer animation is a powerful technique for presenting both natural and artificial stimuli in a controlled fashion, it has inherent limitations. In the present study, entire static images were animated to move back and forth, which provided an element of control across images, but also removed potentially diagnostic factors such as leg movements or postural changes during encounters. Movement and postural cues may be just as, if not more, important than shape and color pattern for visual identification.

Another potential limitation of our study was that a single exemplar of each species and sex was used. As Kroodsma (Kroodsma, 1989) pointed out, this could limit the generalizability of our results. There could be something about the exemplar of *T. sylvana* that we chose, for example, that was more likely to elicit a courtship display than other exemplars of *T. sylvana*.

Additionally, individual males may differ in how they examine and identify other spiders. This seems rather likely given the broad variation in visual inspection times we observed across individuals in each treatment. Perhaps some males do examine a subset of the features making up their conspecific recognition templates, and others do not. Our sample size was limited, so unfortunately, we were only able to make conclusions about general behavioral trends, and not individual variation.

A final limitation of our study was that most of our visual inspection time comparisons were between-group comparisons, which, as was evident with the control female, are not as likely to pick up significant differences as within-group comparisons. Follow-up work using within-group comparisons may bring more clarity to the results presented here.

Evolutionary implications and future directions

Males' conspecific recognition templates were surprisingly coarse, especially for females. In natural environments, misidentifications and resulting maladaptive displays could be fatal. To determine the ecological and evolutionary impact this may have on natural populations, it would be useful to measure how often different salticid species encounter one another in natural environments. Although *L. viridis*, *T. sylvana* and *H. palmarum* have been found on the same tree branches at the same times of year (C.T., unpublished), they may have distinct microhabitat ranges, at the leaf and twig level, that keep them out of each other's way most of

the time. Microhabitat preference evolution can be driven by selection for pre-zygotic reproductive barriers between co-occurring species (i.e. 'reproductive character displacement') (Gröning and Hochkirch, 2008), and may be a more efficient mechanism for species isolation than species recognition templates, which may be more visually and cognitively demanding than microhabitat recognition templates. *Lyssomanes viridis* spends nearly all of its time on the undersides of leaves and spends little time on twigs and branches (C.T., unpublished). Perhaps the converse is true for *T. sylvana* and *H. palmarum*.

Even if similar-looking species do not inhabit completely separated microhabitats, jumping spiders may actively avoid areas of concentrated heterospecific pheromone in order to avoid the dangers inherent to a heterospecific encounter. If so, this would lower an individual's probability of encountering incongruous chemical and visual stimuli. If incongruous stimuli are unlikely to be experienced, then, in the presence of pheromones, coarse recognition templates may work well enough to result in few recognition errors. More stringent templates may require more costly visual and cognitive processing capabilities than are needed to make positive identifications most of the time.

However, even if the above-hypothesized behavioral patterns make stringent visual recognition templates unnecessary for accurate identifications, the coarseness of the template for conspecific females remains surprising. The female forehead spot becomes redder and more clearly defined upon maturation (C.T., unpublished), and is arguably the most distinctive feature of the female's color pattern. This raises the question of what the spot is for, as male *L. viridis*' striking color pattern, exaggerated chelicerae, and courtship and agonistic behaviors suggest that sexual selection has acted primarily on the male phenotype, not the female's (Tedore and Johnsen, 2012). Still, it is possible that the red spot is assessed by males as a signal of quality, and that males prefer females with spots having particular spectral characteristics. Although males rarely fail to court a conspecific female (C.T., unpublished), it is possible that a male would court a higher-quality female more aggressively, and/or would be more likely to fight another male to gain access to her. The red spot could also function as a female–female agonistic signal; however, whether female *L. viridis* compete over territory or resources in nature is unknown. Another possible function of the red spot could be to prevent damaging UV rays from penetrating the translucent cuticle and harming the underlying retinas and brain, which would explain why juveniles also have the spot, albeit a duller version. However, this does not explain why the appearance of the juvenile forehead spot should differ from that of mature females.

In conclusion, our study has raised just as many questions as it has answered, and highlights the potentially important roles that species and sex recognition templates, and their interactions with microhabitat use, may play in the evolutionary dynamics of natural communities. It also brings us closer to understanding how jumping spiders, with their unusual visual system, collect and integrate information from multiple modalities to make decisions. With visual inspection times that lasted anywhere from a fraction of a second to several minutes, as well as coarse recognition templates, it remains a puzzle as to how *L. viridis* manages to consistently discriminate between conspecifics and predators quickly enough to evade predation in natural habitats.

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AUTHOR CONTRIBUTIONS

C.T. planned and executed the experiments, analyzed and interpreted the results, and wrote the manuscript. S.J. participated in analyzing and interpreting the results and in writing the manuscript.

COMPETING INTERESTS

No competing interests declared.

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