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Multimodal signalling: the relative importance of chemical and visual cues from females to the behaviour of male wolf spiders (Lycosidae)

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Many animal signals, especially those important to finding and attracting mates, are multimodal, which means they involve two or more sensory modalities. Among other sensory modalities, the wolf spider, *Pardosa milvina* (Araneae, Lycosidae), uses a variety of chemical and visual information in reproductive activities. Here we report the results of four laboratory experiments in which we explored the effects of visual and chemical information on the behaviour of males. First we established that chemical cues enabled males to find females and that visual cues kept the males' attention focused in the area of the females. Subsequently, we examined the separate and combined effects of visual and chemical cues on the interactions between males competing for virgin or mated females. The behaviour of interacting males revealed that either chemical or visual information was sufficient for them to discriminate between mated and virgin females. Chemical cues elicited more courtship activity and more intense aggressive interactions between males than visual cues. When males were able to see live mated females but were in contact with chemical cues from virgins, aggression was lower and, conversely, when males were able to see live virgin females but were in contact with chemical cues from mated females, aggression increased. We conclude that chemical cues provided males with the most critical discriminatory information. Although the two types of signals are largely redundant, there are some circumstances where they enhance the response of the males and there is an intersignal interaction that allows males to adjust their behaviour when presented with a receptive female.

For effective communication to occur, animals must receive and interpret specific signals against environmental background noise (Guilford & Dawkins 1991; Hebets & Papaj 2005). Although it is easier to explore the specific role of individual signals in isolation, the complexity of animal interactions will typically require information that engages several sensory modalities simultaneously or in sequence (Partan & Marler 1999, 2005; Uetz 2000; Uetz & Roberts 2002; Candolin 2003; Hebets & Papaj 2005). These multimodal signals allow animals to communicate more information with higher signal range, more precise identification of locality, and higher levels of detection in heterogeneous environments where signals have to be filtered from a rich array of alternative sensory input or noise (Partan & Marler 1999, 2005; Hebets & Papaj 2005). Recent reviews discuss the diversity and versatility of multimodal communication and attempt to establish a unifying framework to guide the interpretation of research on this topic (Partan & Marler 1999, 2005; Uetz 2000; Uetz & Roberts 2002; Candolin 2003; Hebets & Papaj 2005).

Sexual displays often involve multimodal signalling presumably because of the complex information that must be exchanged in the two-way interaction between prospective mates that need to identify the viability and genetic quality of one another as potential partners (Candolin 2003; Hebets & Papaj 2005; Partan & Marler 2005). Much of the seminal work on multimodal communication has focused on elaborate male courtship displays that obviously include a variety of signal components (Candolin 2003; Hebets &

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Papaj 2005; Partan & Marler 2005 and references therein). For example, male whitethroats (*Sylvia communis*) perform visual flight displays, which are only successful in attracting females when they are accompanied by specific song elements (Balsby & Dabelsteen 2002). Male courtship in fruit flies (*Drosophila* spp.) involves visual, tactile and chemical signals along with wing vibrations that produce at least two distinct types of acoustic signals (Ewing 1983; Hall 1994; Rybak et al. 2002). Closely related species of wolf spiders in the genus *Schizocosa* differ in their use of unimodal versus multimodal displays (Hebets & Uetz 1999; Uetz 2000; Uetz & Roberts 2002; Hebets 2003; Hebets & Papaj 2005) and, in some species, males shift from emphasizing one sensory component to focusing on another as environmental circumstances change (Taylor et al. 2005a). Indeed, the comprehensive explorations of the male displays in *Schizocosa* spp. serve as models for the study of multimodal communication (Uetz 2000; Uetz & Roberts 2002; Hebets & Papaj 2005).

Fewer studies have explored how males use multimodal communication as they search and possibly compete for receptive females. Males must gather information from females in order to identify and evaluate them as they decide how vigorously to court or fight with other males (Candolin 2003; Guerra & Mason 2005; Jackson et al. 2006). Research on crayfish (*Austropotamobius pallipes*) suggests that males of that species require input to multiple sensory channels in order to recognize females (Acquistapace et al. 2002). Another example revealed that shore crab females (*Carcinus maenas*) release a pheromone that alone elicits mating and defensive behaviour in males, but the contests between males escalate much further when visual and tactile information are also present (Bamber & Naylor 1996; Sneddon et al. 2003). Clearly, multimodal signals from females are critical in guiding male behaviour in these invertebrate taxa. Further explorations of male responses to isolated and composite signals from females in a broader range of taxa will contribute to our understanding of the context, function and distribution of multimodal communication (Uetz 2000; Uetz & Roberts 2002; Hebets & Papaj 2005; Partan & Marler 2005).

The goal of this study was to examine how different types of female cues affect the behaviour of male wolf spiders (Lycosidae). Specifically, we manipulated male access to chemical and visual information from females to determine how it affected the ability of males to find females and influenced the interactions among males. In four experiments, we explored: (1) the relative roles of female visual and chemical cues on male search behaviour, (2) how visual cues from females that differed in mating status (virgin or mated) affected the interactions between two competing males, (3) how chemical cues from females that differed in mating status affected the interactions between two competing males, and (4) how visual and chemical cues work together to affect the interactions between two competing males using a cue conflict design (as recommended in Uetz & Roberts 2002). These experiments were designed to reveal how male wolf spiders use sensory information to find females and respond to male competitors for those same females. We predicted that, in the presence of the appropriate cues from attractive potential mates, males would approach females more quickly, remain in the vicinity of females longer, and fight more vigorously with other males.

To explore how males use multimodal signals from females, we used the wolf spider *Pardosa milvina* (Araneae, Lycosidae) (hereafter referred to as *Pardosa*), a common cursorial spider that is found in disturbed habitats such as agricultural fields in eastern North America (Marshall & Rypstra 1999; Marshall et al. 2002). Adult virgin females produce an airborne pheromone that alone is sufficient to attract males (Searcy et al. 1999). Male *Pardosa* commence a distinctive courtship display of double leg raises and body shakes in the presence of substrate-borne chemical cues, which consist of silk and excreta that remain after females occupy an area (Montgomery 1903; Rypstra et al. 2003; Hoefler et al. 2008). The structure of the genitalia of wolf spiders suggests that they should have first-male sperm priority (Austad 1984), and *Pardosa* males show a distinct preference for virgin over mated females if the female has been allowed to deposit substrate-borne chemical cues in advance (Rypstra et al. 2003). Because this species is highly mobile, it is likely that a male will occasionally encounter chemical cues when he cannot see the female or will catch sight of a female with no chemical information in the vicinity. In addition, since chemical cues may persist after females have vacated, males may also receive visual and chemical information from different females simultaneously. This study examined the manner in which males use these two types of sensory input in their search for females and how that same sensory information from mated or virgin females affects the interactions between potentially competing males.

METHODS

General Laboratory Protocols

Male and female *Pardosa milvina* were collected from corn and soybean fields at Miami University's Ecology Research Center (Oxford, Butler County, OH, U.S.A.) between May and October 2002–2004. We assigned spiders sequential numbers during collection and later used those numbers to randomly assign them to treatments. Spiders collected as adults were maintained in the laboratory for a minimum of 3 weeks before participation in an experiment to acclimatize them and to eliminate differences in hunger. Spiders collected as subadults were held until they reached maturity and then maintained under standard laboratory conditions for an additional 3 weeks after the final moult. We did not attempt to control for the age or mating experience of males because mature males are active in the field throughout the season (Marshall et al. 2002) and so it is likely that males of all ages and levels of experience would be competing for the same females. However, because we knew that the mating status of females was a predictor of their desirability to males (Rypstra et al. 2003), we used it to manipulate female quality. All virgin females moulted to adulthood in the laboratory and were held in isolation from males until the beginning of an experiment. Mated females had produced at least one eggsac in the laboratory or were collected with an eggsac from the field. The eggsacs were gently removed from the spinnerets 1–2 days before the experiment. No spider died during an experimental trial, and after participation, they were either returned to the laboratory population or released into appropriate habitat in the field.

When not used in experiments, *Pardosa* were housed in translucent 150 ml containers (8 cm in diameter, 5 cm walls) with a layer of damp peat moss on the base to provide substrate and moisture. Containers were kept in an environmental chamber at 23 °C on a 13:11 h light:dark cycle. Twice a week, the peat moss was watered and the spiders were fed two crickets (*Acheta domesticus*), each approximately half the size of the spider, or six fruit flies (*Drosophila melanogaster*). Each spider participated in only one trial of one experiment.

Chemical cues were collected by releasing a female into a container with a clean piece of filter paper lining the bottom for a specified time period. As the female moved about the container, she deposited silk and excreta on the filter paper. We refer to these deposited substances as chemical cues, but other substances, such as airborne (olfactory) and substratum-borne chemicals (gustatory) along with tactile cues (thickness, type or density of silk fibres)