Evolutionarily costly courtship displays in a wolf spider: a test of viability indicator theory

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The costs of secondary sexual traits are crucial to our understanding of sexual selection. Although it is broadly accepted that sexual traits are indirectly or directly costly to express, few studies have quantified such costs. Thus, it is unclear if costs are evolutionarily meaningful and to what degree. Costs play a key role in viability indicator models, which assume that 1) the expression of sexual traits reduces the fitness of the trait bearer, 2) sexual trait expression is dependent on condition, and 3) the costs of sexual trait expression are borne differentially, that is, they are less for individuals in good condition. Using 2 syntopic species of wolf spiders, we addressed the importance of direct predation costs on the viability indicator mechanism. Pardosa milvina is a small wolf spider that has conspicuous male courtship behaviors in the form of front leg raises. Hogna helluo is a large species that preys on P. milvina. In laboratory experiments, we discovered that predation risk from H. helluo was higher for courting P. milvina males than noncourting males, male P. milvina manipulated to be in good condition courted at higher rates than males manipulated to be in poor condition, and males in good condition survived predation risk better than males in poor condition. Our study suggests that predation is a significant, evolutionary cost that can satisfy viability indicator mechanism assumptions. Key words: costs, courtship, Lycosidae, predation, sexual selection, spider.

The degree to which sexual traits are expressed is likely to be curbed by the costs of producing them (Darwin 1871). These costs play an important role in viability indicator models and take the form of high energy demands and other mechanisms that tax the physiology of the individual (Kotiaho 2001; Ahlbäken et al. 2005; Sullivan and Kwiatkowski 2007). Most studies of the costs of sexual traits have focused on the first assumption of viability indicator theory. For example, male wolf spiders (Hygrolycosa rubrofasciata) induce to drum (= acoustic sexual signal) at higher rates suffer shorter lifespan (Kotiaho 2000). Also, individuals expressing sexual traits may bear higher predation costs. Predators often exploit the sexual traits of their prey in order to locate them (e.g., Sakuluk and Bellwood 1984; Lima and Dill 1990; Lehmann and Heller 1998; and Sullivan and Kwiatkowski 2007). For instance, brightly colored male guppies (Poecilia reticulata) attract female conspecifics but suffer higher predation levels from blue acara cichlids (Aequidens pulcher) than drab males (Godin and McDonough 2003). Sexual traits may also impose handicaps that limit the signalling’s ability to escape (Andersson 1994). In all cases, if the costs of sexual traits are to be construed as evolutionarily meaningful, it is critical that we demonstrate that their expression reduces survival and/or reproductive success. Although it is broadly accepted that sexual traits incur indirect or direct costs, there is a paucity of studies that have quantified such costs (Kotiaho 2001). To our knowledge, only one published study has attempted to explore whether predation may act as a condition-dependent cost that ensures honest sexual signalling (i.e., satisfies viability indicator theory assumptions): Lindström et al. (2006) discovered that male wolf spiders (H. rubrofasciata) that signalled at high rates incurred greater predation costs when they were not allowed to escape from attacks by experienced pied flycatchers (Ficedula hypoleuca). This study also revealed that the risk of predation increased in proportion to the signalling rate, which does not fulfill the assumption that costs are borne differentially. However, when males were given the opportunity to escape, those with higher mobility were best at...
escaping predation attempts, and initial observations sug-
ggested that mobility and drumming rate were positively corre-
lated. Although condition was not manipulated in the study of
Lindström et al. (2006), their results provide some evidence
that predation costs promote honest signalling and may satisfy
viability indicator model assumptions, but further investiga-
tion is needed.

Using a wolf spider with a conspicuous sexual display, we
tested the assumptions that underlie the viability indicator
model in a laboratory experiment. Specifically, it was our intent
to test if 1) courting males were exposed to higher rates of pre-
dation than noncourting males, 2) the intensity of courtship
was condition dependent such that males in good condition
would court more vigorously than those in poor condition,
and 3) males in good condition were better able to escape pre-
dation than males in poor condition.

MATERIALS AND METHODS

Study system

Wolf spiders (Araneae: Lycosidae) provide good model organ-
isms for studies of the potential costs of sexual signals and the
validity of viability indicator models: they are abundant and
easy to rear; are amenable to manipulative laboratory experi-
ments; and have conspicuous, quantifiable courtship displays.
In the Midwestern United States, the wolf spiders Pardosa
milvina and Hogna helluo are sympatric, cursorial predators that
live in agricultural and early successional habitats, and they
often reach high densities (Marshall et al. 2000, 2002). Pardosa
milvina is a relatively small species (ca. 20–30 mg), and when
in the presence of adult females or female cues, adult males
engage in conspicuous courtship displays that include re-
peated front leg raises (Rypstra et al. 2003). Hogna helluo is
a relatively large species (ca., 300–800 mg) that preys on
P. milvina (Persons et al. 2001). Much is known about the
mating and predatory behaviors of these co-occurring species
(e.g., Persons and Rypstra 2001; Persons et al. 2001, 2002;
Barnes et al. 2002; Walker and Rypstra 2003; Lehmann et al.
2004; Taylor et al. 2005; Bell et al. 2006), and because wolf
spider visual systems are particularly sensitive to movement
(e.g., Rohwer 1996), the leg raises of courting male P. milvina
may heighten their predation risk if they are in close proxim-
ity to H. helluo (e.g., Pruden and Uetz 2004) or another visu-
ally acute predator.

Collection and maintenance of spiders

All P. milvina used in our study were collected as juveniles
from Miami University’s Ecology Research Center (Oxford,
Butler County, OH) during the fall of 2005 and raised to
adulthood in the laboratory. We maintained spiders individu-
ally in 5.5-cm high × 5.5-cm diameter clear, cylindrical plastic
containers adding a 2-cm layer of moistened peat moss as
a substrate to minimize risks of desiccation. Pardosa milvina
were reared on a diet of 2 domestic crickets (2 mm; Acheta
domesticus) twice a week, and peat moss substrates were wa-
tered regularly. Hogna helluo involved in our study were the
first-generation offspring of adult females previously collected
from the Ecology Research Center. We maintained them in
8-cm high × 5.5-cm diameter cylindrical white plastic cups
with clear lids. A 2-cm layer of moistened peat moss was added
as a substrate to allow for their natural burrowing behavior
and to prevent desiccation. We fed H. helluo appropriately
sized domestic house crickets twice a week when they were
juvenile and once a week as adults. All containers with spiders
were housed in an environmental chamber with a relative
humidity near 70%, a photoperiod of 13:11 light:dark, and a
temperature of approximately 25 °C.

General experimental protocol

After the maturation of P. milvina, we randomly assigned
91 adult males to one of 2 condition groups: a poor condition
group (n = 46) and a good condition group (n = 45). Males
assigned to the poor condition group were fed one 2-mm

cricket twice a week, and males assigned to the good condi-
tion group were fed four 2-mm crickets twice a week. We
maintained all male P. milvina on their respective diet regimes
for 3 weeks. Subsequently, we randomly assigned all males to
one of 3 trial groups: a courtship only group, a predation risk
and courtship group, and a predation risk only group. The
courtship only group involved placing an adult male P. milvina
in an arena (described below) with an adult virgin female
P. milvina. Female P. milvina in this group were allowed to
deposit cues (silk, excreta, and associated pheromones) on
a filter paper substrate in the arena for 1 h before trials began,
as substrate-borne cues are important for eliciting male court-
ship behaviors (Rypstra et al. 2003). After 1 h of cue deposi-
tion, we corralled female P. milvina in the center of the arena
under an overturned glass vial (8-cm high × 2-cm diameter)
and introduced the male. Male P. milvina could thus respond
to both visual and putative chemotactile cues from female
conspecifics, but they could not interact with them physically.
The predation risk and courtship group was treated like the
courtship only group; however, we also placed an adult female
H. helluo that had been sated and subsequently starved for
7 days into the arena along with the introduction of the male.
Female P. milvina were protected from predation by H. helluo
under their glass vials throughout the duration of trials, as we
were interested in the potential predation costs of male court-
ship only. The predation risk only group involved placing an
adult male P. milvina in an arena with a starved adult female
H. helluo. An empty, overturned glass vial (8-cm high × 2-cm
diameter) was placed in the center of the arena. We initially
placed male P. milvina and female H. helluo (if present) in
arenas and covered each spider with an overturned plastic vial
(9.5-cm high × 4-cm diameter). The spiders remained under
the vials during a 5-min acclimation period. After this period,
we removed the vials housing male P. milvina and female
H. helluo and allowed them to interact freely for 30 min or
until either predation occurred or the male P. milvina escaped
the arena via a small exit hole in the trial arena (Figure 1).
Spiders were used once in trials. All statistical analyses were
performed using JMP 5.1 statistical software.

Trial arena and data collection

We built and used trial arenas constructed from 2 plastic con-
tainers: a small, inner cylindrical plastic container (7.75-cm
high × 19-cm diameter) fit inside a larger, outer cylindrical
plastic container (8-cm high × 20.5-cm diameter). In the
smaller container, we poured 250 mL of DAP Plaster of Paris
to create smooth, level floors. In addition, we drilled one
0.5-cm diameter circular hole in the side of the smaller con-
tainer such that the base of the hole was flush with the plastic
floor. This hole gave adult male P. milvina but not adult fe-
male H. helluo the opportunity to escape the smaller container
(and enter the larger container) (Figure 1). Before trials be-
gan, we placed a piece of Whatman Filter Paper on the plaster
floor to serve as a substrate. Some arenas were used more than
once during the course of our experiment, and we washed
reused arena containers thoroughly in warm soapy water and
rinsed them with 95% ethanol to remove residual chemical
cues from spiders. We placed arenas in an isolated booth un-
der a Panasonic WV-CP470 video camera located in an envi-
ronmentally controlled room. The camera was connected to
a GOVIDEO R6740 DVD recorder located in a nearby
RESULTS

Male condition

Adult male *P. milvina* assigned to the good condition group were in significantly better condition than males assigned to the poor condition group (*t* ratio = -12.99, *P* < 0.0001, *n* = 30) (Figure 2).

Testing viability indicator theory assumptions

**Assumption 1: sexual traits have fitness costs**

To test the first assumption of viability indicator theory, we compared the survival of male *P. milvina* (good condition and poor condition) assigned to the predation risk and courtship treatment with males assigned to the predation risk only treatment. Males only exhibited leg raises (i.e., expressed the sexual trait) in trials with female conspecifics, and all males expressed the sexual trait in trials with female conspecifics. We made comparisons of survival distributions using a Kaplan–Meier model survival regression analysis. Specifically, we used the nonparametric Kaplan–Meier product limit estimator to test for a significant treatment effect on the median survival distribution. Additional pairwise comparisons were made via the logrank (Mantel–Cox) test.

For male *P. milvina* in poor condition, courtship had a significant effect on predation by *H. helluo* courting males (predation risk and courtship treatment) had significantly lower survivorship than noncourting males (predation risk only treatment) (Mantel–Cox, $\chi^2 = 20.25, P < 0.0001, n = 31$). Similarly, in trials involving males in good condition, courtship had a significant effect on predation: courting males had significantly lower survivorship than noncourting males (Mantel–Cox, $\chi^2 = 10.6, P = 0.0049, n = 31$) (Figure 3).

**Assumption 2: sexual trait expression is condition dependent**

To test the second assumption of viability indicator theory, we compared the courtship rate (leg raises per minute) of male *P. milvina* between males in poor and good condition. We made this comparison in the courtship only treatment and the predation risk and courtship treatment. Because data were not normally distributed, we made comparisons via Mann–Whitney *U* tests.

When no predators were present, male *P. milvina* in good condition courted at significantly higher rates than males in poor condition (Mann–Whitney *U* test, $Z = 4.52, P < 0.0001, n = 29$). Likewise, for males assigned to the predation risk and courtship treatment, males in good condition courted at significantly higher rates than males in poor condition (Mann–Whitney *U* test, $Z = 2.04, P = 0.042, n = 31$) (Figure 4). Interestingly, males in poor condition courted more in the presence of a predator than in treatments with no predator present (Mann–Whitney *U* test, $Z = -2.34, P = 0.0194, n = 31$). This was not the case for males in good condition: those in the courtship only treatment courted at significantly higher rates than males in the predation risk and courtship treatment (Mann–Whitney *U* test, $Z = 2.31, P = 0.021, n = 29$) (Figure 4).
Assumption 3: costs of sexual traits are less for males in good condition

To test the third assumption of viability indicator theory, we compared survival distributions of males in good and poor condition in the predation risk and courtship treatment. As in Assumption 1, we used Kaplan–Meier survival regression analysis to make survivorship comparisons.

Courting male *P. milvina* in good condition had higher survivorship than courting males in poor condition (Mantel–Cox, $\chi^2 = 5.6$, $P = 0.018$, $n = 31$) (Figure 5).

**DISCUSSION**

Results from our study demonstrate that courtship in male *P. milvina* wolf spiders is a clear indicator of quality: courtship reveals information about condition, which is associated with the ability to escape predation from the larger, syntopic wolf spider *H. helluo*. In our study, we discovered that courting male *P. milvina* males suffered greater mortality from predation than noncourting males that were subjected to a real predation risk. This pattern applied to males that were in both good and poor condition. These results satisfy the first assumption of viability indicator models that sexual traits reduce an individual’s fitness and are consistent with previous findings. For example, the acoustically orienting parasitoid fly *Therobia leonidei* uses the trills (= sexual signal) of male bush-cricket males to locate them as hosts (Lehmann and Heller 1998), and the jumping spider *Philippus clarus* uses visual information to hunt courting wolf spiders (*Schizocosa ocreata*) but responds more quickly if multimodal information (visual and seismic sexual signals) is available (Roberts et al. 2007).

Viability indicator models of sexual selection assume that sexually selected traits are evolutionarily costly and are expressed proportionally to the condition of the trait bearer (Zahavi 1977; Johnstone 1995; Kotiaho 2000; Hausken and Hirshleifer 2004). Thus, sexual traits may act as honest indicators of qualities important to the choosier sex. As Kotiaho...
(2001) points out, despite numerous studies suggesting that sexual traits increase risks of predation (e.g., Baker and Bibby 1987; Petrie 1992; Slagwold et al. 1995; Hultia et al. 2005), very few studies have attempted to quantify these costs, and to our knowledge, this study is the first attempt to test whether predation is a significant, evolutionary cost that can satisfy viability indicator mechanism assumptions by simultaneously manipulating condition and the sexual trait. Results from our study indicate that conspicuous courtship behaviors in the form of leg raises in adult male *P. milvina* wolf spiders incur direct fitness costs via predation by *H. helluo*. However, these costs are borne differentially and are less for males in good condition. Our study not only corroborates the generally accepted paradigm that sexual traits are costly but also demonstrates that predation can be a significant, evolutionary cost that satisfies viability indicator mechanism assumptions.

It is important to consider the potentially confounding variable of female *P. milvina* cues (e.g., pheromones, silk, excreta, etc.) that were present in the predation risk and courtship treatment trials but not in trials involving a predation risk only. These cues may have primed the hunting behaviors of *H. helluo* and put male *P. milvina* under a heightened predation risk, as some wolf spiders, including *H. helluo*, have been shown to respond to the chemical cues of their prey (e.g., Persons and Uetz 1996; Persons and Rypstra 2001; Hoefer et al. 2002). However, Persons and Rypstra (2000) demonstrated that *H. helluo* only exhibit foraging biases in behavior for prey for which they have had experience. The *H. helluo* involved in our study were raised on a diet that did not include *P. milvina*, which likely minimized the differences in predation risk between the 2 treatments.

The second prediction of viability indicator models is that sexual trait expression is condition dependent. Results from our study are consistent with this assumption: male *P. milvina* in good condition courted females at significantly higher rates than males in poor condition. This was true for trials in which there was and was not a risk of predation. Males in good condition courted females at significantly higher rates when there was no predation risk from *H. helluo*. This may indicate that males in good condition act more cautiously toward predation risk to compensate for their increased rates of courtship and thus conspicuousness, as has been discovered in field crickets (Hedrick 2000). Conversely, males in poor condition courted females at significantly higher rates when under a real predation risk than when there was no risk of predation. This might suggest that condition affects how males weigh the benefits of mating versus the potential predation costs of courtship, and males in poor condition may take higher predation risks in order to attract prospective mates.

Our results do not entirely agree with previous studies of the effects of predation risk on courtship rate in *P. milvina*. Taylor et al. (2005) demonstrated that male *P. milvina* courtship rate was not affected by predation risk from *H. helluo*; however, the design of their study differed from ours in potentially important ways. Substratum-borne cues from *H. helluo* were used as a proxy for predation, that is, there was not an actual predator present. In addition, *H. helluo* were allowed to deposit cues on filter paper for 24 h before trials began in the study of Taylor et al. (2005), whereas substrate-borne predator cues in our study could have only been laid down during the course of a trial. Results from our study accord with the suggestion of Taylor et al. (2005) that the addition of visual and/or vibrational cues from a real predator may induce modulation of the conspicuous courtship displays of male *P. milvina*, as has been found in other species (e.g., Hedrick 2000). The combined findings that male *P. milvina* in good condition had significantly greater survivorship than males in poor condition and that males in good condition courted at a significantly higher rate than males in poor condition strongly suggest that the costs of male sexual display are less for males in good condition. These findings also satisfy the third assumption of viability indicator models. Thus, male courtship in the form of leg raises is a costly trait that appears to be a reliable, condition-dependent sign of male quality. Reproductive benefits may offset the costs of vigorous courtship, as previous studies have shown that female *P. milvina* are more likely to mate with males that court at high rates (Rypstra et al. 2003).

It is important to acknowledge that our study was entirely laboratory based; thus, demonstrable evidence of evolutionarily meaningful costs of courtship under natural conditions is still needed. However, our study remains relevant to real biological processes. For example, the primary predators of spiders are other spiders (Foelix 1996), and spider body condition (including *P. milvina* condition) ranges widely in the field (Jakob et al. 1996). It is also important to note that we manipulated spider phenotypic and not genotypic condition. Although genetically superior males are, on average, likely to be in better phenotypic condition, this would be a fertile area to explore in future studies.

In conclusion, results from our laboratory study provide compelling evidence for viability indicator models: male *P. milvina* that courted via leg raises were more likely to be preyed on by *H. helluo* than males that did not court, males in good condition courted at higher rates than males in poor condition, and males in good condition had greater survivorship than males in poor condition. To determine with a greater degree of certainty whether leg raises evolved as costly indicators of heritable qualities, it would be valuable to determine if adult females derive indirect benefits from males that naturally court at high rates.

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### REFERENCES


