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NOTE

A General Explanation for the Persistence of Reproductive Interference

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ABSTRACT: Reproductive interference is widespread, despite the theoretical expectation that it should be eliminated by reproductive character displacement (RCD). A possible explanation is that females of sympatric species are too similar phenotypically for males to distinguish between them, resulting in a type of evolutionary dilemma or catch-22 in which reproductive interference persists because male mate recognition (MR) cannot evolve until female phenotypes diverge further, and vice versa. Here we illustrate and test this hypothesis with data on rubyspot damselflies (*Hetaerina* spp.). First, reproductive isolation owing to male MR breaks down with increasing interspecific similarity in female phenotypes. Second, comparing allopatric and sympatric populations yielded no evidence for RCD, suggesting that parallel divergence in female coloration and male MR in allopatry determines the level of reproductive isolation on secondary contact. Whenever reproductive isolation depends on male MR and females of sympatric species are phenotypically similar, the evolutionary catch-22 hypothesis offers an explanation for the persistence of reproductive interference.

Keywords: reproductive interference, character displacement, local mate competition, Odonata, mate recognition, evolutionary catch-22.

Introduction

When phenotypically similar species come into contact, interspecific sexual interactions—known as reproductive interference—can occur (Gröning and Hochkirch 2008). To varying degrees, different forms of reproductive interference—such as misdirected courtship, heterospecific mating attempts, and hybridization—carry fitness costs for individuals of one or all species involved (Gröning and Hoch-

kirch 2008). In the face of such costs, current theory predicts local extinction of one or more species (i.e., reproductive exclusion; Kuno 1992; Liou and Price 1994; Hochkirch et al. 2007; Gröning and Hochkirch 2008; Pfennig and Pfennig 2012; Kishi and Nakazawa 2013; Grether et al. 2017; but see Ruokolainen and Hanski 2016) or evolutionary divergence in traits involved in mate recognition (i.e., reproductive character displacement; Brown and Wilson 1956; Hochkirch et al. 2007; Burdfield-Steel and Shuker 2011; Pfennig and Pfennig 2012; Grether et al. 2017). Yet reproductive interference is widespread in animals (Gröning and Hochkirch 2008; Shuker and Burdfield-Steel 2017), even among species that appear to coexist stably (e.g., insects: Hochkirch et al. 2007; Drury et al. 2015a, 2015b; Shuker et al. 2015; spiders: Taylor et al. 2017; nematodes: Ting and Cutter 2018; amphibians: Pfennig 2003; birds: Veen et al. 2010). Why does reproductive interference persist in spite of its fitness costs (Takakura et al. 2015; Grether et al. 2017)?

We propose that reproductive interference often persists because of an evolutionary catch-22 arising from interspecific sexual conflict.¹ Reproductive interference is likely to carry a net cost for females, but for males, the cost of missed conspecific mating opportunities is often greater than the cost of mating with heterospecific females (Shuker et al. 2015). The catch-22 is that if females of sympatric species are too similar phenotypically for males to profitably attempt to distinguish between them, reproductive interference will persist indefinitely because there is no selection for divergence in male mate recognition (MR) and, consequently, no selection on females to diverge phenotypically. Even if males have an incipient ability to distinguish conspecific and heterospecific females, the resulting divergent selection on female phenotypes might be too weak to override stabilizing or convergent selection, because female phe-

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1. An evolutionary catch-22 is a kind of dilemma in which A cannot evolve until B evolves, and vice versa (Maynard Smith 1983).

notypes that are potentially useful for male MR (e.g., coloration) are likely targets of selection in other contexts (e.g., predation), and closely related sympatric species can be expected to adapt in similar ways to their common environment (e.g., Gomez and Théry 2004).

The evolutionary catch-22 hypothesis could be rejected for a particular taxon by showing that reproductive isolation resulting from enhanced male MR can evolve in sympatry, while counterevidence would support the hypothesis (fig. 1). The rubyspot damselfly genus *Hetaerina* is an example of a taxon in which reproductive interference occurs at high levels between most but not all sympatric species (Drury et al. 2015a, 2015b). Unlike some other odonates, there is no courtship in *Hetaerina*; reproductive interactions begin with males clasping females in flight. Building on previous phenotype manipulation experiments and comparative analyses (Drury et al. 2015a, 2015b), we show here that species differences in female wing coloration are predic-

tive of the level of reproductive isolation caused by male MR in a clade-wide analysis, confirming that reproductive interference arises, at least in part, from similarity in female phenotypes. One species, *Hetaerina titia*, stands out, both for being highly variable in wing coloration and for usually experiencing low levels of reproductive interference (Drury et al. 2015a, 2015b). To determine how the low levels of reproductive interference evolved, we measured male MR in *H. titia* and two sympatric congeners (*Hetaerina occisa*, *Hetaerina americana*) across a network of allopatric and sympatric sites. If male MR were enhanced in sympatry, this would imply that male MR evolved via reproductive character displacement (RCD) and directly contradict the evolutionary catch-22 hypothesis (fig. 1). Instead, we find that male MR does not differ between sympatry and allopatry, which suggests that male MR diverged prior to secondary contact rather than through RCD, supporting the evolutionary catch-22 hypothesis.

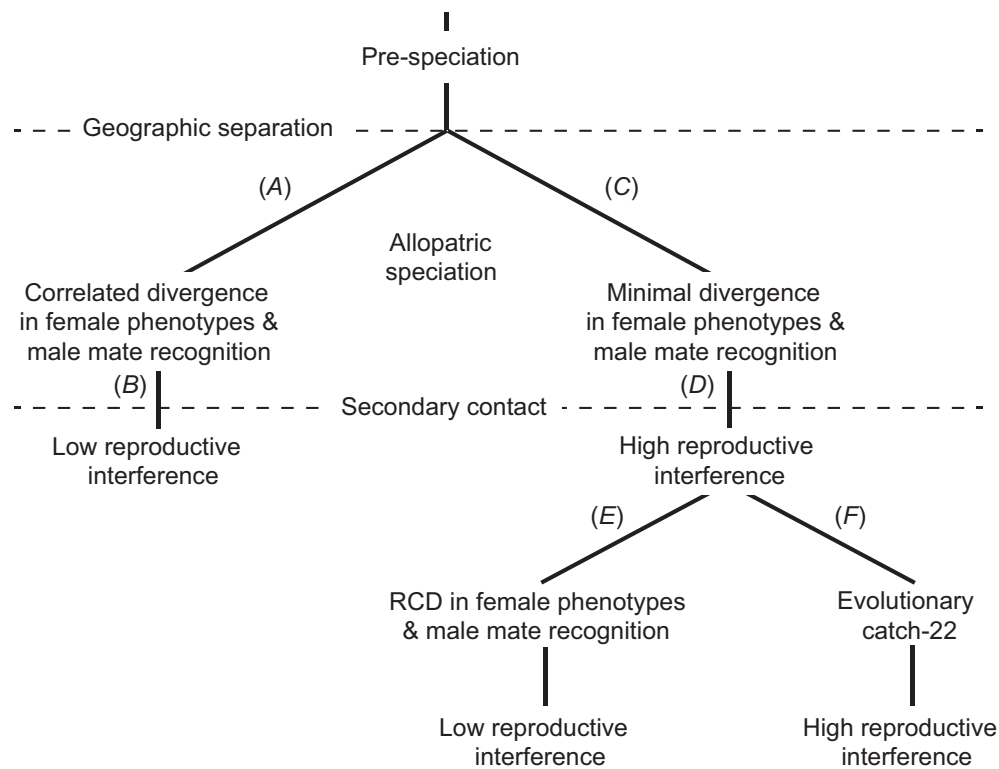


Figure 1: Schematic diagram showing two evolutionary routes from speciation in allopatry to low reproductive interference in sympatry. Species either diverge in female phenotypes and male mate recognition in allopatry (A) and are reproductively isolated at the time of secondary contact (B), or they diverge minimally in allopatry (C), experience high levels of reproductive interference on secondary contact (D), and subsequently diverge in female phenotypes and male mate recognition through reproductive character displacement (RCD; E). Alternatively, species with high reproductive interference might be caught in an evolutionary catch-22 arising from interspecific sexual conflict (F). Evidence for RCD would show that species can escape from high reproductive interference and thereby contradict the catch-22 hypothesis, while evidence against RCD would support the catch-22 hypothesis.

Methods

Male MR and Female Wing Coloration

To test the core assumption that similarity in female phenotypes contributes to reproductive interference, we measured male sexual responses to conspecific and heterospecific females at 16 sites in the United States, Mexico, and Costa Rica where two or more species of *Hetaerina* occur in sympatry (10 species, 13 species pairs) between 2010 and 2017 (table A1; tables A1–A9 are available online). Following established protocols (Anderson and Grether 2010b; Anderson et al. 2011), we marked all males along the stream transects (~100–250 m) and identified territorial males (i.e., males resighted at the same location, ± 1.5 m, on consecutive days; Anderson and Grether 2010b). We then presented tethered females to territorial males for ~5 s and recorded male responses; males were considered to respond sexually if they clasped or attempted to clasp the female. When a male did not clasp a tethered female on the first presentation, we presented her a second time. One conspecific female and one female of each sympatric congener were presented sequentially, and we systematically alternated the presentation order, with at least 2 min separating the presentations. We considered a trial to be successful if a male responded sexually to either female. We calculated an index of reproductive isolation due to male MR for each species in each species pair:

$$\frac{S_c - S_h}{S_c + S_h},$$

where S_c is the proportion of successful trials in which males responded sexually to conspecific females, and S_h is the proportion of successful trials in which males responded sexually to heterospecific females (Stalker 1942; Martin and Mendelson 2016).² This index was not affected by the number of males for which we measured male MR (table A2; mean n heterospecific trials = 23.77 [range = 7–64]; mean n conspecific trials = 24.94 [range = 7–64]).

We measured species differences in female wing coloration from digital photography ($n = 1,101$ photographs; mean \pm SD per species per site = 32.28 ± 20.03 ; $n = 16$ sites) and reflectance spectroscopy ($n = 505$ females; mean \pm SD per species per site = 20.32 ± 7.87 ; $n = 12$ sites), using established protocols (Drury et al. 2015b; see also table A3).

We computed Spearman's rank correlations between the reproductive isolation indexes and several measures of species differences in female wing coloration for each species at each site (table A3). To account for phylogenetic noninde-

pendence and the pairwise structure of the data, we computed P values using permutation and phylogenetic simulation methods (appendix, available online; Drury et al. 2015b, 2018).

Testing for RCD in Male MR

To test for RCD of male MR in *Hetaerina titia*, conspecific and *Hetaerina americana* females were presented to *H. titia* territory holders in sympatry and allopatry, following the tethering protocol described above. To test for RCD of male MR in *Hetaerina occisa* and *H. americana*, territory holders of those species were presented with conspecific females, and either *H. titia* females (in the case of *H. occisa*) or conspecific females with experimentally darkened wings (to mimic female *H. titia*; conducted with both *H. americana* and *H. occisa*) in allopatry and sympatry (appendix; Drury et al. 2015a, 2015b). For the tests in allopatry with heterospecific females, live heterospecific females were transported to the sites. Translocated females were kept in the shade to avoid overheating during transfer, and most trials were conducted with transported females within hours of their capture, though for a few trials, we used females collected the previous day who were still flying strongly. In our RCD tests, allopatric populations were between 5 and 551 km away from paired sympatric sites and, with one exception, were in separate drainages (table A1).

We tested for RCD by fitting logistic regression models to male responses and testing for an interaction between site type (i.e., sympatry/allopatry) and female treatment (e.g., conspecific/heterospecific). We fitted GLMMs with male identity as a random effect in the R package glmmADMB (Fournier et al. 2012), though when male responses were too dichotomous to reliably fit GLMMs, we fitted logistic regressions using bayesglm (Gelman and Su 2016).

Results

The level of reproductive isolation resulting from male MR in *Hetaerina* correlated with species differences in female wing coloration (figs. 2A, 2B, A1; table A5); with increasing interspecific differences in female wing lightness, there is increasing reproductive isolation from male MR. Data and code for running analyses are deposited in the Dryad Digital Repository (<https://dx.doi.org/10.5061/dryad.63mk0ks>; Drury et al. 2019).³

In two species pairs with pronounced reproductive isolation due to male MR in sympatry (*Hetaerina titia*–*Hetaerina americana*, *H. titia*–*Hetaerina occisa*), equally high levels of

2. Use of the term “mate recognition,” based on behavioral responses, is standard in evolutionary ecology but differs from the definitions used in signal detection theory and psychology (Mendelson 2015).

3. Code that appears in *The American Naturalist* is provided as a convenience to readers. It has not necessarily been tested as part of peer review.

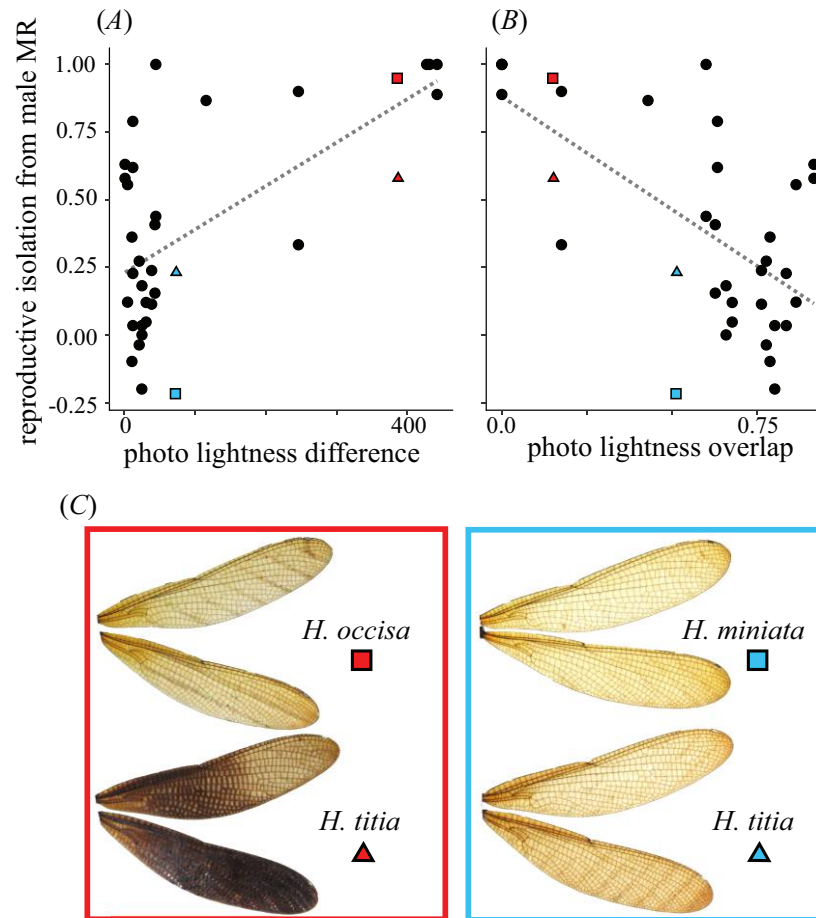


Figure 2: Reproductive isolation resulting from male mate recognition correlates with species differences in female wing lightness, measured as the Euclidean difference in lightness values (mean $\rho = 0.54$, $P < .001$; A) or the pairwise overlap of photographic lightness density curves at each site (mean $\rho = -0.64$, $P < .001$; B). Each point depicts the responses of males of a particular species at a particular site. Red and blue points highlight the species comparisons for which females are shown in panel C.

reproductive isolation occur in allopatry (fig. 3A, 3B; table 1). Likewise, males of the light-winged species, *H. americana* and *H. occisa*, rarely respond sexually to conspecific females with experimentally darkened wings, whether in sympatry or allopatry with *H. titia* (fig. 3C, 3D; table 1). Although *H. occisa* males tended to respond to darkened females at a higher rate in sympatry compared to allopatry (fig. 3D), this is owing to an overall increase in male responses in sympatry (i.e., the main effect of site type; table 1) rather than an increase in responses toward darkened females per se. The interaction between site type and female treatment was not significant (table 1). Permutation tests demonstrate that the nonsignificant interaction terms are statistically robust (fig. A3). Removing the one within-drainage comparison, for which genetic isolation is likely low (tables A6–A8; fig. A4), does not qualitatively change our results (table A9).

Discussion

Reproductive isolation due to male mate recognition can be predicted from phenotypic similarity of heterospecific and conspecific females in *Hetaerina* (fig. 2), which in combination with experimental manipulations of female wing coloration (Drury et al. 2015a, 2015b) confirms that reproductive interference arises, at least in part, from similarity in female phenotypes. In two pairs of species with minimal reproductive interference (Drury et al. 2015b), our sympatry-allopatry comparisons yielded no evidence that reproductive interference has been reduced by RCD in male MR (fig. 3). Indeed, in three of our sympatry-allopatry comparisons, males tended to be more likely to respond sexually to heterospecific or experimentally darkened females in sympatry compared to allopatry (table 1; fig. 3B–3D)—a trend

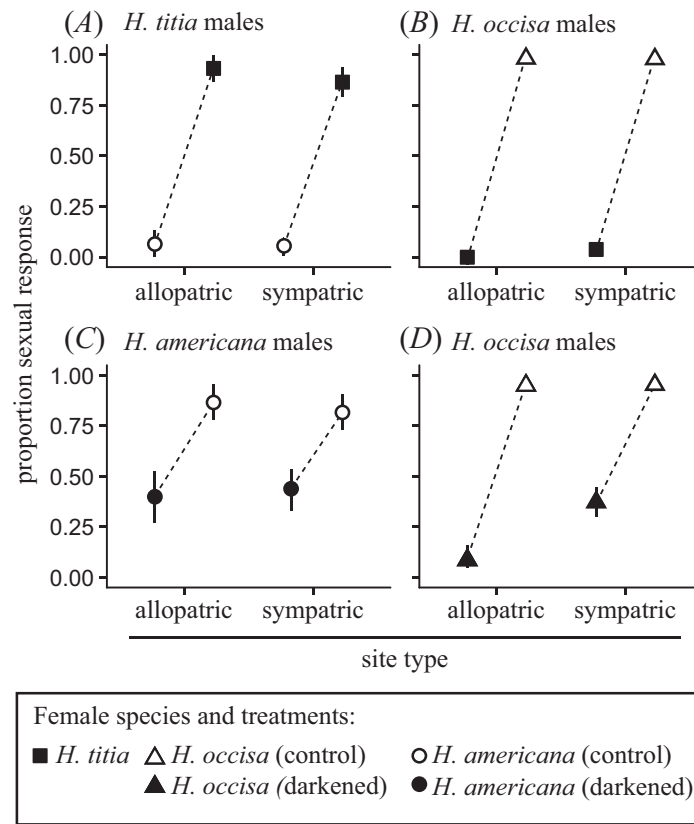


Figure 3: No evidence for reproductive character displacement in male mate recognition. When males discriminate between conspecific and heterospecific female phenotypes, they do so equally well in sympatry and allopatry. Responses (shown as the proportion of successful trials with sexual responses \pm SE) in sympatry and allopatry of *Hetaerina titia* males to *Hetaerina americana* and *H. titia* females (A), *Hetaerina occisa* males to *H. titia* and *H. occisa* females (B), *H. americana* males to control and experimentally darkened conspecific females (C), and *H. occisa* males to control and experimentally darkened conspecific females (D). Apparently, sympatric populations of these species were already reproductively isolated on secondary contact (see fig. 1).

that goes in the opposite direction of the reproductive character displacement hypothesis's prediction.

We cannot rule out that all five allopatric populations are recently derived from sympatric populations, but this is a less parsimonious scenario and more difficult to reconcile with observed high levels of genetic differentiation and isolation-by-distance among populations of *Hetaerina americana* (tables A6–A8; fig. A4) than the alternative that divergence in male MR evolved prior to secondary contact. Moreover, repeat visits to sites in the breeding season shows that the species present and even their relative abundance is stable from year to year (Anderson and Grether 2010a). Thus, we infer sympatric *Hetaerina* species with high levels of reproductive interference are probably caught in an evolutionary catch-22 from which they cannot escape through RCD (fig. 1). Previous research demonstrates that interspecific territoriality is widespread between sympatric species of *Hetaerina* damselflies. Theoretical modeling and field experimentation support the hypothesis that such interspecific territoriality

has likely been maintained by selection in response to extensive reproductive interference (Drury et al. 2015b). Yet, the persistence of reproductive interference itself was previously largely unexplained (Gröning and Hochkirch 2008; Grether et al. 2017).

The evolutionary catch-22 hypothesis is a viable explanation for the persistence of reproductive interference in several other clades in which males exhibit a limited ability to discriminate between females of closely related species (e.g., *Tetranychus* spider mites [Sato et al. 2014], lygaeid bugs [Shuker et al. 2015], *Habronattus* jumping spiders [Taylor et al. 2017; reviewed in Gröning and Hochkirch 2008]). On a broader phylogenetic scale, this catch-22 hypothesis predicts that reproductive interference should be more prevalent where the cost to males of missed conspecific mating opportunities is high relative to the cost of interacting with heterospecific females. We would expect to find high levels of reproductive interference where males invest little in each mating attempt and not in species with prolonged courtship

Table 1: Analyses of male sexual responses toward tethered females in sympatry and allopatry

| Experiment, male species (<i>n</i> males), term | Est. | SE | <i>z</i> -value | <i>P</i> |
|--|-------|------|-----------------|----------|
| Translocation: ^a | | | | |
| <i>Hetaerina titia</i> (37): | | | | |
| Intercept | -2.45 | .82 | -2.97 | .003 |
| Female species (<i>H. titia</i>) | 4.79 | 1.08 | 4.43 | <.001 |
| Site type (sympatry) | -.47 | .99 | -.48 | .63 |
| Female species × site type | -.02 | 1.20 | -.01 | .99 |
| <i>Hetaerina occisa</i> (70): | | | | |
| Intercept | 4.13 | 1.29 | -3.19 | .001 |
| Female species (<i>H. titia</i>) | -8.37 | 1.71 | -4.90 | <.001 |
| Site type (sympatry) | -.13 | 1.29 | -.10 | .92 |
| Female species × site type | .40 | 1.60 | .25 | .80 |
| Wing darkening: ^b | | | | |
| <i>Hetaerina americana</i> (38): | | | | |
| Intercept | -.41 | .53 | -.77 | .44 |
| Female treatment (control) | 2.27 | .93 | 2.46 | .014 |
| Site type (sympatry) | .14 | .67 | .21 | .83 |
| Female treatment × site type | -.46 | 1.16 | -.40 | .69 |
| <i>H. occisa</i> (72): | | | | |
| Intercept | -2.16 | .61 | -3.54 | <.001 |
| Female treatment (control) | 5.49 | 1.19 | 4.63 | <.001 |
| Site type (sympatry) | 1.64 | .69 | 2.38 | .02 |
| Female treatment × site type | -1.95 | 1.43 | -1.37 | .17 |

^a Logistic regression models fit using bayesglm in R.

^b Mixed-effect logistic regression models with random intercept terms for male IDs, fit using glmmADMB in R.

or nuptial gifts. For instance, some research on *Calopteryx* damselflies revealed geographic shifts in male MR consistent with RCD having acted on male MR in sympatry (Waage 1975; Wellenreuther et al. 2010). Given that males in this genus perform elaborate courtship displays, low levels of reproductive interference might result from selection against misdirected courtship. However, other studies on *Calopteryx* species have variously found strong sexual isolation due to male MR in sympatry and allopatry (Svensson et al. 2014) or persistent reproductive interference between sympatric taxa (Tynkkynen et al. 2008; Svensson et al. 2016).

In many taxa, whether reproductive interference occurs depends on male MR and associated female traits, but historically the focus of RCD and speciation research has been on female MR and male traits (Mendelson et al. 2018; Tinghitella et al. 2018). Theoretical expectations of either RCD or reproductive exclusion follow from models where the costs of reproductive interference are high for both sexes, and as a result, selection on male signal traits and female mating preferences should favor interspecific divergence. Yet for males of many species, the costs of missed mating opportunities may be higher than the costs of reproductive interference (Takakura et al. 2015), leading to persistent reproductive interference and precluding the ability for selection to favor divergence in female traits. Given the central role of behavioral interference in the dynamics of species inter-

actions (Grether et al. 2017), our approach underscores the importance of accounting for male mating decisions and female phenotypes in models of species coexistence and range expansion.

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Data and Code Availability

Data and code used in analyses are available in the Dryad Digital Repository (<http://dx.doi.org/10.5061/dryad.63mk0ks>; Drury et al. 2019). Sequence data are available on GenBank (see table A4 for accession numbers).

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A male *Hetaerina vulnerata* and an experimentally tethered female *Hetaerina cruentata* in tandem. Photo credit: Andrew Chao.