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SCIENTIFIC NOTE

Reproductive Interference Between Native and Introduced Lady Beetles (Coleoptera: Coccinellidae) in Puerto Rico

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Lady beetles, ladybugs, or ladybird beetles (Coccinellidae) and their larvae feed on soft-bodied arthropods such as aphids, mealybugs, scales, whiteflies (Hemiptera), and mites (Arachnida) (Obrycki and Kring 1998), among others. Because of their diet, they are widely used as biocontrol agents in diverse ecosystems from agricultural to forests (Obrycki and Kring 1998; Tavares *et al*. 2014). In Puerto Rico, the lady beetle fauna consists of 53 described species, seven of which have been introduced as biological control agents (Segarra-Carmona *et al*. 2021). Introduced and native lady beetles sometimes exploit similar prey; therefore, their habitats can overlap. Their proximity can result in interspecific interactions that could potentially hinder a desired biocontrol goal or have adverse effects on the fitness of either species; however, there is much to learn about this.

Interspecific interactions can be of several types, and some of their effects have been studied. For example, "competition" for similar prey (Elliott *et al*. 1996; Soares and Serpa 2007) and "cannibalism" of larvae of the native species by the exotic (Katsanis *et al*. 2013) can reduce native species' populations. For instance, potential interspecific interactions may be involved in the reduction of the once naturally abundant nine-spotted lady beetle (*Coccinella novemnotata* Herbst), which has become extremely rare across its native range in North America. One such interaction, the reproductive interference (*i.e*., interspecific mating) with the seven-spotted lady beetle (*Coccinella septempunctata* Linnaeus), has been observed in laboratory experiments (Stellwag 2015). Reproductive interference occurs when individuals of two different species engage in reproductive activities that reduce the fitness of any of the involved species (Gröning and Hochkirch 2008). For example, reproductive interference by *Harmonia axyridis* (Pallas) reduced mating opportunities of *Harmonia yedoensis* (Takizawa) with conspecifics in the laboratory (Noriyuki *et al.* 2012). Therefore, a similar interspecific interaction occurring in nature could reduce a species' population, and thus its role in the ecosystem. Different types of interspecific reproductive interference include interspecific rivalry, interspecific courtship, interspecific mating or mating attempts, among others (Burdfield-Steel and Shuker 2011). Because reproductive interference has the potential of negatively affecting outcomes of important and time-sensitive biocontrol operations, it is in the best interest of biological control efforts to contemplate potential issues arising from it.

In Puerto Rico, the species *Coelophora inaequalis* (Fabricius) was introduced to control the yellow sugar cane aphid, *Sipha flava* (Forbes) (Aphididae) (Bowling *et al*. 2016; Okosun *et al*. 2021). This lady beetle has spread to other crops in which the native species, *Cycloneda sanguinea limbifer* (Casey), occurs naturally (Miskimen 1970). This creates opportunities for interspecific interactions to occur between the two species. Here I report previously undescribed intergeneric mating attempts (a type of reproductive interference) occurring in nature between these two species and also between the native and *Olla v-nigrum* (Mulsant), whose presence in Puerto Rico is of uncertain origin (Segarra-Carmona *et al*. 2021). Two observations were recorded photographically in 2008 and extended for two to four minutes. Observations took place in northwest Puerto Rico (approximately 18°22′N; 66°50′W; 315 m ASL) before the start of the rainy season. Both interactions happened on the same citrus tree (*Citrus* × *sinensis* (L.) Osbeck; Rutaceae) that was infested with aphids (*Toxoptera* sp.; Aphididae) at the time. The first interaction was photographed on 4 May. This involved a male *C. s. limbifer* (male copulatory organ visible in Fig. 1a) attempting to mate with an apparent female *O. v-nigrum* (Fig. 1a). I observed the second interaction on 10 May, this involved an apparent male *C. s. limbifer* but the mating attempt with an apparent female of *C. inaequalis*

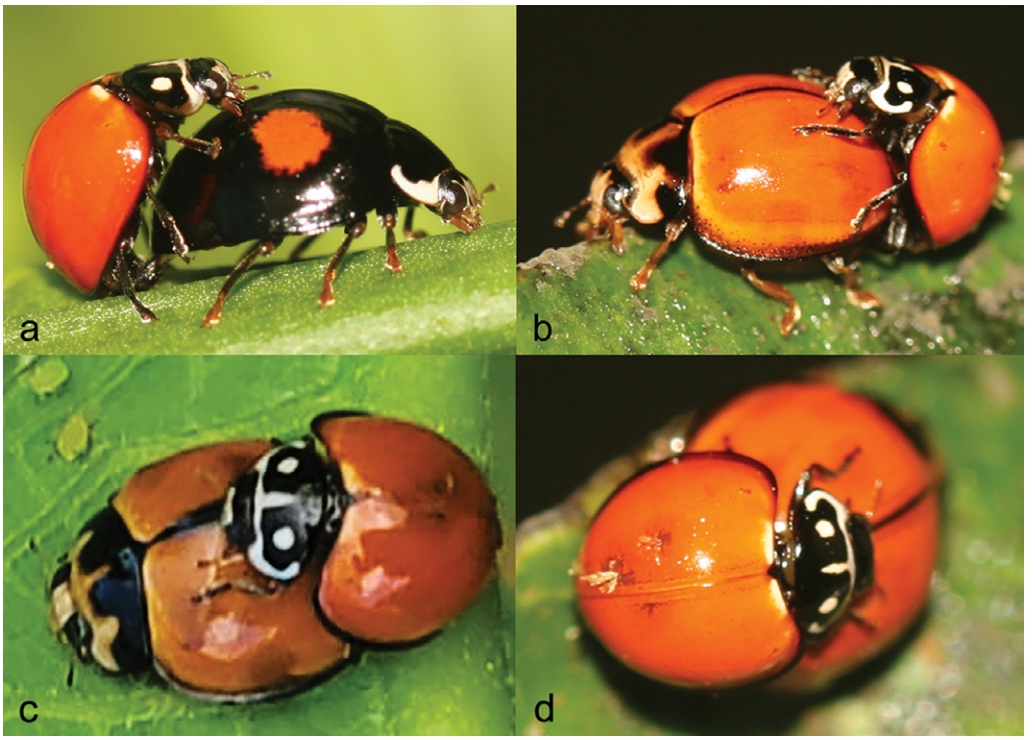

Fig. 1. Male *Cycloneda sanguinea limbifer* attempting to mate heterogeneric lady beetle females. a) With female *Olla v-nigrum*; b–d) With female *Coelophora inaequalis*. Laboulbeniales thalli can be seen on the elytra of two *C. s. sanguinea* (a and b, d).

(immaculate morph) (Fig. 1b). Given similarities between *C. s. limbifer* and *C. inaequalis*, I did not recognize it as being an interspecific mating attempt until reviewing the photo folder containing the first observation. At the time of reviewing the manuscript, a photo of a third observation involving an intergeneric mating attempt between *C. s. limbifer* and *C. inaequalis* (Fig. 1c) was shared on an online forum, and the observer provided data about the observation. The activity occurred close to the date of the two previous observations, on 20 April 2023. In this instance, the mating pair was on an avocado tree, *Persea americana* Miller (Lauraceae), in southwestern Puerto Rico (approximately 18°09′N; 67°04′W; 90 m ASL). This observation also lasted about two minutes. None of the individuals observed were collected.

Reproductive interference through interspecific mating has been reported in nature in Japan (Nakano 1985) and Europe, where these have occurred between lady beetles of different genera (Majerus 1997). However, this has not been reported in nature in the Nearctic or Neotropical regions. Instances of this behavior may pass unnoticed since identification of some lady beetle species can be challenging due their multiple and contrasting patterns and color morphs. The two apparent female species discussed here are good examples of this. In Puerto Rico, the two orange-spotted black morph of *O. v-nigrum* is reported almost exclusively (Segarra-Carmona *et al*. 2021), but the species has another four known and greatly contrasting morphs that vary not only in spot patterns but also in background color. Meanwhile, *C. inaequalis* varies in the degree of darkness of its elytral spots, from nearly immaculate to having deep black spots, and in their number and pattern.

It is possible these mating attempts occurred because males of *C. s. limbifer* either fail to distinguish females of other lady beetle species or, if able, attempt to mate with heterospecific females for yet unknown reasons. Identifying the mechanism by which *C. s. limbifer* finds mates would help explain why intergeneric mating is attempted. Stimuli used by lady beetles to find mates include visual and olfactory (Hodek and Ceryngier 2000), but which is/are used by *C. s. limbifer* have not been studied. It is probable that visual cues were not used by the *C. s. limbifer* male attempting to mate with the two orange-spotted black females of *O. v. nigrum*, but whether it can visually confuse an orange

immaculate female *C. inaequalis* should be investigated. Alternatively, an olfactory mate-finding system in *C. sanguinea* remains unknown, but its use of olfactory cues for prey searching suggests the species may use smell for other purposes. *Cycloneda sanguinea* was attracted to a combination of healthy prey and host plant smells (Heit *et al*. 2008) and rejected a competitor's smell (Sarmento *et al*. 2007); however, no differences were found in its response to either aphid prey or to the aphid's plant host (Heit *et al*. 2011). Discoveries of sexual pheromone synthesis by female Asian lady beetle, *H. axyridis*, and other species, suggest sexual communication is used by lady beetles (Fassotte *et al*. 2014). We need to identify the semiochemicals used by the three species discussed here to learn whether they can recognize heterospecific/heterogeneric females as a result of the semiochemicals emitted.

Since neither visual nor pheromone cues yet explain the observed intergeneric mating attempts presented here, other less common alternatives may be considered, for example, behavioral changes mediated by viruses or parasites such as wasps or fungi, which remains largely unexplored in lady beetles. From my observations, two male *C. s. limbifer* carried ectosymbiotic fungi in the Laboulbeniales (Ascomycota) on their elytra (Figs. 1a, b, d). Although the fungal specimens were not examined, they resemble fungi in the cosmopolitan and lady beetle specialist genus *Hesperomyces* Thaxter (Laboulbeniaceae) by characteristics such as location on host, color, general aspect (Haelewaters *et al.* 2020), and apparent damage to the elytra that can be observed (Fig. 1d). Species in the genus *Hesperomyces* have not yet been documented in Puerto Rico, but *H. axyridis*, a prolific vector of *Hesperomyces harmonia* Haelew. & De Kesel, has been suspected to be in the island since 2014 and was confirmed in 2017 (Hiller and Haelwaters 2019; Segarra-Carmona *et al*. 2021). Furthermore, *Hesperomyces virescens* Thaxter has been reported from most of the world, affecting 30 species of lady beetles including *C. sanguinea* and *O. v. nigrum*. Dissemination of *Hesperomyces* spp. by *H. axyridis* is facilitated by their winter aggregation behavior, but it is also considered a sexually-transmitted "disease" (Nalepa *et al.* 2007). Although behavioral changes in hosts are not known to be caused by *Hesperomyces* spp., they can impair their fitness (Knapp *et al*. 2022) and increase their susceptibility to infection by entomopathogens (Haelewaters *et al.* 2020). Moreover, other fungi in the order Laboulbeniales have been found to cause behavioral changes in ants (Báthori *et al*. 2017). Therefore, whether *Hesperomyces* spp. have direct effects such as behavioral changes due to physiological control or indirect by their production of semiochemicals attractive to their lady beetle hosts remains a fertile area for investigation.

Due to the observations presented here and their potential significance (conserving native species biodiversity and reducing disease transmission), it would be worthy to consider examining reproductive interference in closely related taxa (perhaps to tribe, as seen here) when considering biocontrol plans involving lady beetles. Also, as reproductive interference can also result in indirect effects such as the transmission of diseases and parasites (Hurst *et al*. 1995; Knapp *et al.* 2022; Nalepa and Weir 2007; Webberley *et al*. 2006), these should also be considered. The apparent increase in the dissemination of *Hesperomyces* spp. between lady beetle species and into novel areas should be explored under the umbrella of climate change.

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