bioRxiv preprint doi: https://doi.org/10.1101/740506; this version posted August 20, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Lack of phenotypic variation in larval utilization of pea aphids in populations of the ladybeetle *Hippodamia convergens*

Christy Grenier[*], Bryce Summerhays[*], Ryan Cartmill[*], Tanairi Martinez[*], Roxane Saisho[*], Alexander Rothenberg[*], Jerrika Scott[*+], John J Obrycki[$], Arun Sethuraman[*]

[*] Department of Biological Sciences, California State University San Marcos, San Marcos, California

[+] Department of Biology, Benedict College, Columbia, South Carolina

[$] Department of Entomology, University of Kentucky, Lexington, Kentucky

bioRxiv preprint doi: https://doi.org/10.1101/740506; this version posted August 20, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Abstract

The convergent lady beetle (*Hippodamia convergens*) is a generalist natural enemy that is utilized extensively in augmentative biological control across the United States. Recent studies have pointed to both genetic and phenotypic differences in Western (California) versus Eastern (Kansas) populations of the species. Here we investigate phenotypic differences in their utilization of pea aphids in (a) Eastern versus Western populations, (b) Hybrid Eastern and Western populations versus their progenitor populations, and (c) within population competition rates in Eastern, Western, and Hybrid populations. Across eight replicate treatments, we find no phenotypic differences ($P > 0.2$), contradicting previous genetic and behavioral studies. Hybrid F1 populations however show an overall greater percentage weight gain, potentially indicative of a hybrid vigor effect, and greater fitness of augmented populations of the species.

bioRxiv preprint doi: https://doi.org/10.1101/740506; this version posted August 20, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Introduction

Ladybird beetles (also called ladybugs, Coleoptera: Coccinellidae) are commonly utilized as natural enemies against infestation of aphids, whiteflies, and scales across the world (Roy and Wajnberg 2008). Most coccinellid beetles are multivoltine, producing two or more broods within a year (Koch and Hutchison 2003). In North America, *Hippodamia convergens* is the most abundant native species of coccinellids used in both introduction and augmentative biological control (Bjørnson, S 2008). Recent population genetic studies of *H. convergens* across their range in the continental United States have revealed the presence of at least two structured geographic populations (termed Western and Eastern populations - Sethuraman et al., 2015). These populations have also previously been shown to have differences in developmental histories, overwintering behavior, and reproductive diapause, but are able to hybridize with each other without any known reproductive barriers (Obrycki and Tauber 1982, Obrycki et al 2001). The Western population disperses into the Rocky Mountains to form large overwintering aggregations (Wheeler and Ring 2014). These large concentrations of adults make the Western population easily susceptible to unregulated collections, which are then sold to farmers or home gardeners across the United States (Obrycki and Kring 1998). These commercially sold beetles are released annually for augmentative biological control across the United States (see Sethuraman et al. 2015). However, no comparative studies of the utilization of aphids by Western and Eastern populations of *H. convergens* have been conducted. This type of study is needed to quantify the potential levels of aphid biological control resulting from augmentative releases of the Western populations of *H. convergens*.

Biological control, while providing effective control of agricultural pests, comes at the cost of, or is affected by several non-target effects. Modern agricultural practices often utilize

bioRxiv preprint doi: https://doi.org/10.1101/740506; this version posted August 20, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

pesticides against crop pests, but have adverse consequences for the pests and other beneficial arthropods which are utilized in biological control (Biondi et al., 2012a, 2012b, Geiger et al., 2010), and contribute to global arthropod decline (Potts et al., 2010, Benton et al., 2002, Hallmann et al., 2017). Transportation and augmentation of *H. convergens* populations has also led to the movement and spread of arthropod pathogens and parasitoids (Bjørnson, S 2008). Despite their extensive utility in augmentative biological control, little is known about the effectiveness of transporting Western collected populations of *H. convergens* throughout the United States. Studies have been conducted in California to document the effects of augmentative releases of *H. convergens* (Flint et al., 1995; Flint and Dreistadt, 2005). *Hippodamia convergens* within the designated Western and Eastern populations in the United States have likely adapted to varying natural conditions, pathogens and parasitoids cycles. Augmenting populations by bringing Western and Eastern populations together to create hybrids can also potentially increase the fitness of the hybrid population, a phenomenon that is commonly described as `hybrid vigor' (Seko et al., 2012).

The objective of this study is to understand the effectiveness of human mediated augmentation of predatory *H. convergens* from the Western population to the Eastern population, and how potential competition between Eastern and Western populations might differentially affect rates of biological control. Using both the Eastern and Western population of *H. convergens* lady beetles found in the United States, as well as hybrid population reared with mating pairs of Eastern and Western beetle populations, we address the following questions: (1) Do Eastern and Western populations differ in their effectiveness of utilization of pea aphids?, (2) Are hybrids of Eastern and Western populations more effective at the utilization of pea aphids than their progenitor populations?, and (3) Is there competition within Eastern, Western, and

bioRxiv preprint doi: https://doi.org/10.1101/740506; this version posted August 20, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

hybrid populations? We address these questions by (1) assessing the percentage weight gain (as a proxy for fitness) of Eastern and Western larvae when placed individually on aphid bearing plants, as well as assessing the populations' percentage weight gain when both populations are placed on the same plant, (2) raising hybrid population larvae to assess their percentage weight gain when individually placed on an aphid bearing plant, as well as two larvae on the same plant, compared to the percentage weight gain of the Eastern and Western populations under the same conditions, and (3) assessing the percentage weight gain of Eastern, Western, and hybrid larvae when two individuals of the same population are placed on the same plant.

Methods

H. convergens were raised from field collected beetle egg masses from Kansas (JP Michaud), representing the Eastern Population of the species. The Western population of *H. convergens* were field collected from adult aggregations on Palomar Mountain in San Diego County in Southern California. Beetles were raised on frozen or live pea aphids, *Acyrthosiphon pisum*, which were reared on fava bean plants (*Vicia faba)*, in a greenhouse at the California State University San Marcos, San Marcos, CA, with an average high temperature of about 27.7 °C and average low temperature of 16.3 °C from January to May 2019. To obtain hybrid and inbred beetle larvae, the following crosses were made: (1) Eastern population virgin female crossed with Eastern population male, (2) Eastern population virgin female crossed with a Western population male, (3) Western population virgin female crossed with a Western population male, (4) and Western population virgin female crossed with an Eastern population male. Mating pairs were allowed 48 hours to mate, after which the males were separated, and females were fed pea aphids *ad libitum*, and allowed 48 hours to lay egg masses. Once the egg

bioRxiv preprint doi: https://doi.org/10.1101/740506; this version posted August 20, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

masses were laid, females were separated, and egg masses were collected in preparation for the competition assays.

To assess for competition and biocontrol efficacy, a common-garden setup was utilized. A fava bean plant (~10 cm in height, 2 week old sapling, with 6-8 leaves) was placed in a 2 liter plastic bottle with a cut-out black mesh window. Larvae from the crossing experiments were separated into individual cups, and starved for 24 hours prior to the beginning of our assay. After 24hrs, ~0.150g of aphids (approximately 50 aphids) were placed inside each bottle and allowed approximately 3-12 hours to settle and infect the fava bean plant. Thereon, 3rd instar larvae of similar size (to allow commencement of the experiments at the same developmental stage) were weighed using an analytical balance and then placed inside the following treatment bottles: 1) 2 Eastern population larvae - to assess within Eastern population competition, 2) 2 Western population larvae - to assess within Western population competition, 3) 2 East-West hybrid larvae - to assess competition in augmented populations, 4) 1 Western population larva with 1 Eastern population larva - to assess competition between Eastern and Western populations which were painted with acrylic paint to determine the individuals. Individuals were repainted following molts . Three controls were also set up: 5) 1 Eastern population larva, 6) 1 Western population larva, and 7) 1 East-West hybrid larva. Larvae were then weighed every other day with an analytical balance until pupation, where the weight of the pupa would be the final weight recorded (approximately 8 days). On the fourth day, fava bean plants inside the bottles were watered, and another approximate 0.150g of pea aphids were placed inside each bottle to ensure each fava bean plant still had aphids, and that all larvae had *ad libitum* access to food (This ensures that larvae will survive – but likely reduced any competition – but the larval survival data would address this. The experiments were repeated eight replicates from March-May 2019.

bioRxiv preprint doi: https://doi.org/10.1101/740506; this version posted August 20, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Results from treatments where larvae had gone missing or died were eliminated from statistical analyses of weight gain.

Statistical Analyses

Performance of Eastern,Western, and hybrid populations of *H. convergens* was assessed using the beetles' percentage weight gain (final minus initial weight, divided by initial weight) as a proxy for fitness. A mean percentage weight gain was calculated, when it was not possible to distinguish individuals in conspecific larval replicates. All statistical analyses were performed using JMP v.14. Tests of normality were conducted on each dataset, and transformations were performed when applicable depending on the distribution of data. A series of ANOVAs were performed (Table 1) to test the following hypotheses: 1) The Western population of *H. convergens* is better at pea aphid utilization than the Eastern population, 2) Due to hybrid vigor, the Hybrid population will be better at utilizing pea aphids than both the Eastern and Western population, and 3) in the competition assay, a Western *H. convergens* larva will show greater weight gain compared to an Eastern *H. convergens* larva.

Results

In all treatments, *H. convergens* larvae molted successfully and pupated into adults. The percentage weight gain of the larvae when individually placed on pea aphid bearing plants was not different between the Eastern, Western, and Hybrid populations (d.f.=2, F=0.2476, and P= 0.7829, Table 1). The percentage weight gain of an Eastern individual larva was not statistically significant when compared to the percentage weight gain of a single Western individual larva (d.f.=1, F=0, P=0.9991,Table 1). Furthermore, the percentage weight gain of a Hybrid individual vs the percentage weight gain of both Eastern individuals and Western individuals was also

bioRxiv preprint doi: https://doi.org/10.1101/740506; this version posted August 20, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

similar (d.f = 1, F=1.789,P=0.204; and d.f.= 1, F= 1.2812, and P=0.2781, Table 1). The percent weight gain between the Eastern individual versus the Western individual bore no statistical significance (d.f.= 1, F= 0.3724, P= 0.5515), indicating no significant competition during this experiment. The percentage weight gain when individual Eastern, Hybrid, or Western larvae, compared to two Eastern, Hybrid, or Western larvae were similar (d.f=1, F=0.048, P=0.8297; d.f.= 1, F= 1.3887, P= 0.2615; and d.f.=1, F= 0.0076, P= 0.9317, Table 1). There was no statistical significance when comparing the percentage weight gain of when two individual Eastern larvae compared to one Eastern and Western larvae (d.f=1, F= 1.7671, P= 0.205). The same is also true when comparing the percentage weight gain of when two Western larvae were compared to one Eastern and one Western larvae (d.f= 1, F= 0.3787, P= 0.5482, Table 1). Comparison of the percentage weight gain of two Hybrids to two Eastern larvae, or two Western larvae are not statistically significant (d.f= 1, F=0.0575, P= 0.814; d.f.= 1, F= 0.0256, P= 0.8752,Table 1). Finally, a 2-way ANOVA showed no statistical significance when comparing the percent weight gain of 1 individual larvae vs 2 larvae and eastern vs western larvae (d.f.= 3, F= 0.0937 P= 0.9629, Table 1).

Discussion

Several species of terrestrial arthropods are utilized extensively as natural enemies against common agricultural pests and invasive species, and are estimated to result in billions of dollars in agricultural savings across the globe (Coombs et al 1996, Huang et al., 2018). Within the United States, beneficial insects provide over 50 billion dollars worth of services and 4.5 billion is contributed to biological control organisms known as natural enemies (Landis et al., 2008). Natural enemies can have lasting effects by establishing populations in the introduced ranges for long term recurring utilization of crop pests (Enkerli et al., 2004). Often times

bioRxiv preprint doi: https://doi.org/10.1101/740506; this version posted August 20, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

biological control involves introducing non-native species into a new range (Dodd, Alan 1959), which interact with a diverse array of intra- and inter-specific competitors (Evans 1991). Quantifying their effectiveness is therefore of great importance to biological control programs (Tauber and Tauber 1975, Evans 1991).

Within the United States, it is common practice to transfer populations of Western *H. convergens* to the Eastern United States to control aphid pest infestations. This augmentative biological control can lead to hybridization of Eastern and Western populations (Sethuraman et al., 2015). Hybridization of lady beetles could also increase the fitness of hybrid populations, and therefore increase their effectiveness for use in biological control. To asssess the utilization of aphids by native, and hybrid beetles, we perform a series of crosses and measure the percentage increase in weight gain as a measure of fitness. Percentage weight gains following competition between Eastern, Western, and hybrid individuals however, show that there was no significant difference ($P > 0.2$, Table 1) among, or between populations.

Larger lady beetles generally have greater choice of prey species, potentially of higher nutrient value (Sloggett 2008). Larger females have also been shown to have higher fecundity, and fitness (Kajita and Evans 2010). However, our assessment of larger beetles (hybrids from our treatments, Fig. 1) shows no such phenotypic difference in utilization of pea aphids, compared to smaller, progenitor beetles. Our findings also conflict with previous studies that conclude that there are significant phenotypic differences between Eastern and Western populations of *H. convergens* in the United States, that may affect their effectiveness as a biocontrol agent (Obrycki et al., 2001). For instance, evidence of differences in photoperiodic responses have been observed in beetle populations that have not adapted to their local environment, which can result in slower developmental cycles when compared to populations that are native to the area

bioRxiv preprint doi: https://doi.org/10.1101/740506; this version posted August 20, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

(Obrycki et al., 2018). This response to photoperiods has also been shown to be heritable, indicating that augmentation and importation may also affect the ability for future generations of the introduced population to compete with native populations (Reznik et al., 2017). Similarly, differences in temperature regimes in newer environments could also lead to a difference in reproductive diapause between populations, that can cause introduced populations to develop at a slower rate than the native population (Wang et al., 2013).

Our study showed that in general, hybrid beetles have a larger percentage weight gain (Fig 1A). However, the differences in percentage weight gains were not statistically significant ($P > 0.2$) when compared to their parent populations (Table 1). The apparently increased percentage weight gain may be attributed to hybrid vigor, wherein hybrid populations are known to exhibit physical traits and behavioral phenotypes that confer greater fitness than their progenitor populations (Seko et al., 2012, Li et al., 2018). Additionally, we acknowledge that there is a chance that hybridization could have occurred in one of our progenitor populations prior to the start of our experiment, which could have contributed to their apparent similarities in relative depredation rates. Both Eastern and Western populations are known to migrate over large geographical distances, however it is unknown if mating occurs between the populations during migration, and if the populations return to their previous locations **(**Sethuraman et al., 2015). This could however be elucidated by analyzing the population genetics of our Eastern, Western, and Hybrid populations, which is beyond the scope of this study.

The Western, Eastern and hybrids of *H. convergens* did not show any significant differences ($P > 0.2$) in percentage weight gain when interacting with other individuals of the same population. Interactions of lady beetle larvae did not bring any changes in weight when given excess aphids, though when beetle larvae were being starved for 24 hours together, the

bioRxiv preprint doi: https://doi.org/10.1101/740506; this version posted August 20, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

larger and older instars were found to feed on younger instars. Intraspecies/guild predation is well documented in lady beetles, especially when there is a large size difference between larvae and adults on larva or eggs (Bayoumy et al., 2015, Agarwala and Dixon 1992). We paired most of the larvae to be in their third instar stage, and were approximately similar in size and weight. However, some larvae escaped, or disappeared from the tent, putatively indicating intra-specific depredation. This data was subsequently removed from the study so as to not bias our statistical analyses.

We did not find any significant differences between our Eastern, Western, or Hybrid population of beetles, as individuals, or paired, in their effectiveness of utilization of the aphid crop pests. These experiments were conducted in a greenhouse with semi-regulated temperatures in Southern California, an environment which does not mirror the colder environment of the Eastern Region of the United States. A previous study of thermal requirements for development of lady beetles showed that some species of coccinellids develop earlier in colder seasons while some species develop faster in warmer temperatures (Obrycki and Tauber 1981), and that developmental rates differ between Eastern and Western Population of *H. convergens* at colder temperatures, but are similar in warmer temperatures (Obrycki and Tauber 1982). The non-significant differences between Eastern and Western individuals could hence also be attributed to testing in higher, Western temperatures in Southern California. Further studies should thus measure the rates of utilization of aphids in cooler temperatures that mimic the Eastern Region of the United States environment and temperatures. These studies would allow a better understanding of the environmental effects on introduced, or augmented populations of *H. convergens* in the colder versus warmer regions of the Unites States.

bioRxiv preprint doi: https://doi.org/10.1101/740506; this version posted August 20, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Acknowledgments

This work was funded by USDA grant #2017-06423 to George Vourlitis and Arun Sethuraman. We would like to acknowledge our collaborator, Dr. JP Michaud for field collecting and shipping the Kansas (Eastern) population of *H. convergens*. RS, RC were funded by a CSUSM Summer Scholars fellowship, and JS was funded by an NSF REU grant #1852189 to Betsy Read and Arun Sethuraman.

Author Contributions

JJO, AS, BS conceptualized the experiments, BS, CG, TM, and RC performed all the experiments, and RS, AR, and JS performed all the statistical analyses.

References

1. Agarwala, Basant K., and Anthony FG Dixon. "Laboratory study of cannibalism and interspecific predation in ladybirds." Ecological Entomology 17.4 (1992): 303-309
2. Benton, Tim G., et al. "Linking agricultural practice to insect and bird populations: a historical study over three decades." Journal of Applied Ecology 39.4 (2002): 673-687.
3. Biondi, Antonio, et al. "The non-target impact of spinosyns on beneficial arthropods." Pest management science 68.12 (2012): 1523-1536.
4. Biondi, Antonio, et al. "Using organic-certified rather than synthetic pesticides may not be safer for biological control agents: selectivity and side effects of 14 pesticides on the predator Orius laevigatus." Chemosphere 87.7 (2012): 803-812.

bioRxiv preprint doi: https://doi.org/10.1101/740506; this version posted August 20, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

5. Bjørnson, S. "Natural enemies of the convergent lady beetle, Hippodamia convergens Guérin-Méneville: their inadvertent importation and potential significance for augmentative biological control." Biological Control 44.3 (2008): 305-311.

6. Dodd, Alan P. "The biological control of prickly pear in Australia." Biogeography and ecology in Australia. Springer, Dordrecht, 1959. 565-577.

7. Egg cannibalism and its life history consequences vary with life stage, sex, and reproductive status in Hippodamia convergens (Coleoptera: Coccinellidae)." Journal of economic entomology 108.4 (2015): 1665-1674.

8. Enkerli, Jürg, Franco Widmer, and Siegfried Keller. "Long-term field persistence of Beauveria brongniartii strains applied as biocontrol agents against European cockchafer larvae in Switzerland." Biological Control 29.1 (2004): 115-123.

9. Evans, Edward W. "Intra versus interspecific interactions of ladybeetles (Coleoptera: Coccinellidae) attacking aphids."*Oecologia* 87.3 (1991): 401-408.

10. Flint, M., et al. "Lady beetle release controls aphids on potted plants." *California Agriculture* 49.2 (1995): 5-8.

11. Flint, Mary Louise, and Steve H. Dreistadt. "Interactions among convergent lady beetle (Hippodamia convergens) releases, aphid populations, and rose cultivar." *Biological Control* 34.1 (2005): 38-46.

12. Geiger, Flavia, et al. "Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland." Basic and Applied Ecology 11.2 (2010): 97-105.

13. Hallmann, Caspar A., et al. "More than 75 percent decline over 27 years in total flying insect biomass in protected areas." PloS one 12.10 (2017): e0185809.

bioRxiv preprint doi: https://doi.org/10.1101/740506; this version posted August 20, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

14. Huang, Jikun, et al. "Uncovering the economic value of natural enemies and true costs of chemical insecticides to cotton farmers in China." Environmental Research Letters 13.6 (2018): 064027.

15. Koch, R. L., and W. D. Hutchison. "Phenology and blacklight trapping of the multicolored Asian lady beetle (Coleoptera: Coccinellidae) in a Minnesota agricultural landscape." *Journal of Entomological Science* 38.3 (2003): 477-480.

16. Landis, Douglas A., et al. "Increasing corn for biofuel production reduces biocontrol services in agricultural landscapes." Proceedings of the National Academy of Sciences 105.51 (2008): 20552-20557.

17. Li, Hao-Sen, et al. "Population admixture can enhance establishment success of the introduced biological control agent Cryptolaemus montrouzieri." *BMC evolutionary biology*18.1 (2018): 36.

18. Obrycki, John J., and Maurice J. Tauber. "Thermal requirements for development of Hippodamia convergens (Coleoptera: Coccinellidae)." Annals of the Entomological Society of America 75.6 (1982): 678-683.

19. Obrycki, John J., and Timothy J. Kring. "Predaceous Coccinellidae in biological control." *Annual review of entomology* 43.1 (1998): 295-321.

20. Obrycki, John J., et al. "Comparative studies of three populations of the lady beetle predator Hippodamia convergens (Coleoptera: Coccinellidae)." *Florida Entomologist*(2001): 55-62.

21. Obrycki, John J., et al. "Photoperiodic Induction of Adult Diapause in North American Populations of the Convergent Lady Beetle (Coleoptera: Coccinellidae)." *Environmental entomology* 47.6 (2018): 1596-1600.

bioRxiv preprint doi: https://doi.org/10.1101/740506; this version posted August 20, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

22. Potts, Simon G., et al. "Global pollinator declines: trends, impacts and drivers." Trends in ecology & evolution 25.6 (2010): 345-353.

23. Reznik, S. Ya, et al. "On the photoperiodic induction of diapause in first generation hybrids of Harmonia axyridis (Pall.)(Coleoptera, Coccinellidae)." *Russian Journal of Genetics: Applied Research* 7.6 (2017): 717-720.

24. Roy, Helen, and Eric Wajnberg. "From biological control to invasion: the ladybird Harmonia axyridis as a model species." *BioControl* 53.1 (2008): 1-4.

25. Seko, Tomokazu, Takahisa Miyatake, and Kazuki Miura. "Assessment of hybrid vigor between flightless lines to restore survival and reproductive characteristics in the ladybird beetle Harmonia axyridis." *BioControl* 57.1 (2012): 85-93.

26. Sethuraman, Arun, Fredric J. Janzen, and John Obrycki. "Population genetics of the predatory lady beetle Hippodamia convergens." Biological Control 84 (2015): 1-10.

27. Sloggett, John J. "Weighty matters: body size, diet and specialization in aphidophagous ladybird beetles (Coleoptera: Coccinellidae)." *European Journal of Entomology* 105.3 (2008).

28. Tauber, Maurice J., and Catherine A. Tauber. "Criteria for selecting Chrysopa carnea biotypes for biological control: Adult dietary requirements." *The Canadian Entomologist*107.6 (1975): 589-595.

29. Wang, Su, et al. "Effect of temperature and photoperiod on the development, reproduction, and predation of the predatory ladybird Cheilomenes sexmaculata (Coleoptera: Coccinellidae)." *Journal of economic entomology* 106.6 (2013): 2621-2629.

30. Wheeler, Christopher A., and Ring T. Cardé. "Following in their footprints: cuticular hydrocarbons as overwintering aggregation site markers in Hippodamia convergens." Journal of chemical ecology 40.5 (2014): 418-428.

bioRxiv preprint doi: https://doi.org/10.1101/740506; this version posted August 20, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

31. Yukie Kajita, Edward W. Evans, Relationships of Body Size, Fecundity, and Invasion Success among Predatory Lady Beetles (Coleoptera: Coccinellidae) Inhabiting Alfalfa Fields, *Annals of the Entomological Society of America*, Volume 103, Issue 5, 1 September 2010, Pages 750–756, https://doi.org/10.1603/AN10071

bioRxiv preprint doi: https://doi.org/10.1101/740506; this version posted August 20, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Tables and Figures

Table 1. Two-way and One-way ANOVAs results when comparing percentage weight gain of individual, two larvae, Eastern, Western, and Hybrid larvae of *H. convergens*. Percentage weight gain (Final weight – Initial Weight/Initial Weight) was used for all statistical tests, and data were transformed and normalized when applicable for every statistical test. All tests were performed using JMP v.14.

Treatment	df	MS	F	P
1 Individual vs 2 individuals + Eastern vs Western	3	0.054948	0.0937	0.9629
1 Eastern vs 2 Eastern	1	0.27826	0.048	0.8297
1 Western vs 2 Western	1	0.004678	0.0076	0.9317
1 Hybrid vs 1 Eastern vs 1 Western	2	0.14485	0.2476	0.7829
Eastern vs Western when placed on same plant	1	1.10776	0.3724	0.5515
1 Hybrid vs 2 Hybrid	1	0.203849	1.3887	0.2615
2 Eastern vs Eastern and Western on same plant	1	7.96651	1.7671	0.205
2 Hybrid vs 2 Eastern	1	0.3351	0.0575	0.814
2 Hybrid vs 2 Western	1	0.004536	0.0256	0.8752
1 Eastern vs 1 Western	1	6.25E-06	0	0.9991

bioRxiv preprint doi: https://doi.org/10.1101/740506; this version posted August 20, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

1 Hybrid vs 1 Eastern	1	0.509465	1.789	0.204
2 Hybrid vs 1 Western	1	0.150319	1.2812	0.2781
Eastern and Western on the same plant vs 2 Western	1	0.059941	0.3787	0.5482

bioRxiv preprint doi: https://doi.org/10.1101/740506; this version posted August 20, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

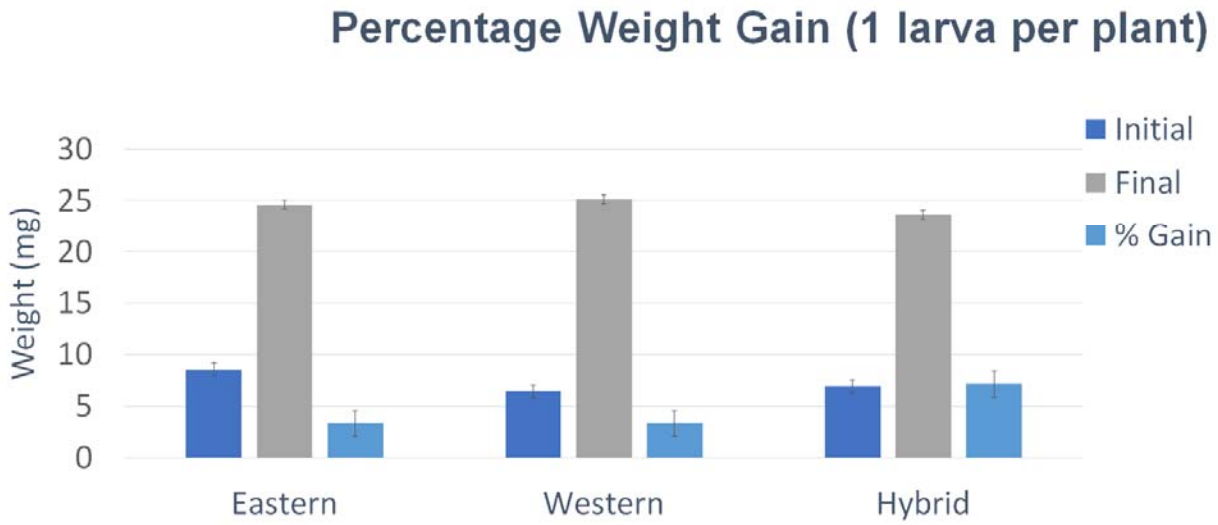

Fig. 1 Weight in mg (initial, final), and percentage weight gain from aphid utilization in Eastern, Western, and Hybrid populations of *Hippodamia convergens* when an individual larva was placed per plant, across eight replicate treatments.

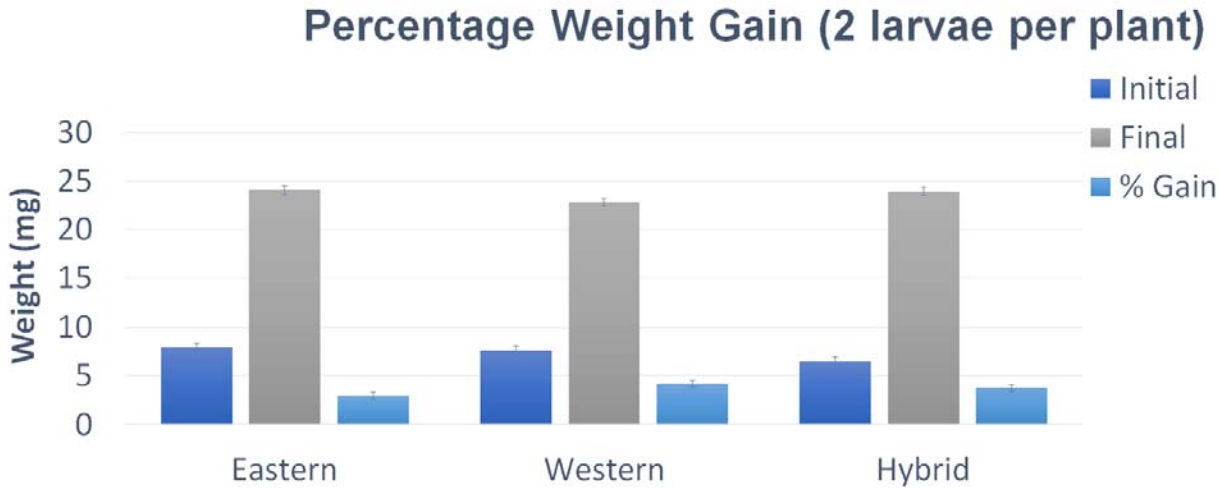

Fig. 2 Mean weight in mg (initial, final), and percentage mean weight gain from aphid utilization in Eastern, Western, and Hybrid populations of *Hippodamia convergens* when two larvae were placed per plant, across eight replicate treatments.