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# Behavioral Ecology of an Invasive Species: Habitat and Mate Preference(s) in *Drosophila Suzukii*

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BEHAVIORAL ECOLOGY OF AN INVASIVE SPECIES:  
HABITAT AND MATE PREFERENCE(S) IN *DROSOPHILA SUZUKII*

By

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## ABSTRACT

The invasive Spotted-Wing fruit fly, *Drosophila suzukii*, has inflicted substantial economic losses to the soft-fruit agriculture industry worldwide due to the ability of females in this species to use a large, serrated ovipositor to cut the fruit's skin and lay eggs directly into the mesocarp of ripening fruit. Once the eggs hatch, larvae consume the fruit flesh, ultimately leaving the fruit unmarketable. This species parasitizes numerous commercial fruit types (including blueberries, blackberries, strawberries, raspberries and occasionally grapes) as well as fruits from a variety of wild plant species. Since fruit types vary in their nutritional composition, as well as their spatial and temporal availability, this suggests that *D. suzukii* experiences considerable environmental heterogeneity. The environment can have a large influence on the development and evolution of morphological, physiological, and behavioural phenotypes and, thus, can have significant implications for individual variation and population growth and change over time. Thus to better understand success of this invasive species we have examined the behavioural and developmental and reproductive performance of *D. suzukii* as it relates to their local environment. Specifically we focused on the role of the nutritional developmental history (NDH), which can either hinder or support offspring growth and survival, and may be an important factor when selecting habitats. Secondly, as the juvenile NDH has the potential to greatly influence adult phenotypes, and consequently individual reproductive potential, we also examined if NDH influenced the operation of sexual selection. Here we report evidence that NDH is an important factor for *D. suzukii* females when choosing oviposition sites, and can have dramatic consequences for offspring development, mate-choice, offspring fitness, and ultimately population growth. Information on these two subject areas (habitat and mate choice) is of great potential importance to bettering future management strategies (e.g. by manipulating fly numbers based on host

preference and reproductive strategies), which are currently limited by lack of knowledge on the biology of this species.

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## **CHAPTER 1**

### THE SIGNIFICANCE OF PREFERENCES IN *DROSOPHILA SUZUKII*

#### **Background**

*Drosophila suzukii* is a species of fruit fly originating in Asia (Kanzawa 1936) that has spread in the last dozen years to large portions of North America, South America and Europe, where it is considered an invasive pest species of significant economic threat (reviewed by Asplen *et al.* 2015). In *D. suzukii*, females oviposit into ripe or ripening soft-fruits, rather than rotting or damaged fruits, which is more typical of other *Drosophila spp.* (Walsh *et al.* 2011), thanks to the presence of a large, serrated, ovipositor, which is used to cut the fruit's skin (Atallah *et al.* 2014). Once hatched, the larvae consume the fruit tissue, creating soft and sunken areas, while fungal, bacterial, yeast and other infections render the fruit unmarketable (Walsh *et al.* 2011). Unfortunately, a number of commercial fruit types including blueberries, blackberries, strawberries, raspberries and occasionally grapes are parasitized by this fruit fly (Bellamy *et al.* 2011; Lee *et al.* 2011), and, as a result, soft-fruit agriculture has suffered losses of up to 80% (USDA NASS 2009) with Bolda *et al.* (2010) estimating an economic loss of \$500 million to California, Oregon and Washington, annually. As *D. suzukii* appears capable of rapidly invading vast areas of landscape, possibly facilitated by the utilization of native and ornamental fruiting plants (Heimpel *et al.* 2010; Walsh *et al.* 2011), there is also the potential for continued increasing costs in the future. For instance, *D. suzukii* was confirmed to inhabit the entire length of California through British Columbia (Bolda *et al.* 2010) following its first detection in Hawaii less than two decades earlier (Kaneshiro 1983). By 2010, flies had also been detected in Utah, Louisiana, North Carolina, South Carolina, Wisconsin, Michigan, Alberta, Manitoba, Ontario and Quebec (Burrack 2011; Davis 2011; Hamilton 2011; Isaacs 2010; Saguez 2013).

Currently, farmers rely extensively on insecticides and baited traps as a means of management of *D. suzukii* (Cini *et al.* 2012), both of which have proven to be largely ineffective at controlling infestations (Burrack *et al.* 2011; Cini *et al.* 2012; Iglesias *et al.* 2014; Lee *et al.* 2013). These strategies are expensive and come with the risk of impacting non-target biota, through excessive chemical applications (Cini *et al.* 2012). Not surprisingly, then, there have been many scientific studies conducted to determine better control methods (Timmeren & Isaacs 2013; Swoboda-Bhattarai & Burrack 2014; Lee *et al.* 2013). However, most studies remain within the realm of designing traps (e.g., varying bait recipes or trap colours), implementing insecticide protocols, and/or determining potential host susceptibility in various fruits. Meanwhile, the role that habitat and mate choice may have on life history variation has been largely overlooked. This is a surprising oversight given the importance of the environment and sexual selection as the means by which a wide variety of morphologies, physiologies, and behaviours have evolved (Andersson 1994). The research that makes up my thesis focuses on trying to understand how habitat and mate choice influences the life history of *D. suzukii* – specifically as it relates to individual fitness. By investigating these two areas, we may gain an understanding into the ways in which *D. suzukii* individuals attempt to acquire fitness benefits (e.g., through increased fecundity, or through advantageous habitat choice). Knowledge in these areas may be significant for designing innovative management strategies capable of disrupting the key components of this species’ success, especially since, given that the North American environment offers many novel hosts and climates, making good fitness-related choices may be central to its ability to adapt and thrive in unfamiliar landscapes.

## *Home “Sweet” Home:*

### **The importance of understanding habitat choice and its consequences in *D. suzukii***

Habitat choice is an important aspect of many species' ecology, and as such, has the potential to provide crucial insight into infestation intensity in *D. suzukii*. For instance, if flies have strong preferences for certain host fruits, they may choose to disperse widely in their environment, settling only when a desirable host site is found, while if they do not have strong preferences, their dispersal range may be more confined. Thus the movement of flies could greatly shift population distribution over time and space as a direct result of their habitat needs. In current models that estimate *D. suzukii* population growth, the positioning of flies is assumed to be static (e.g. Wiman *et al.* 2014), and the potential effects of immigration and/or emigration to control and manage are not considered. Given the vast geographic area *D. suzukii* has covered in the past two decades, it seems short-sighted to discount exploration as a potential significant trait in this species. Additionally, *D. suzukii* has been observed to travel among different crops in a mixed orchard setting (Harris *et al.* 2014) and other *Drosophila* sp. are known to travel in search of favorable habitat, traversing long distances in short periods of time (e.g. up to 676 km in 15 hours; Coyne *et al.* 1982; Jones *et al.* 1981). Knowledge on habitat choice in *D. suzukii* may thus be an important factor for determining population size among different crops.

Habitat preferences in *D. suzukii* may also help to explain how this species has succeeded and thrived in novel environments. For instance, in some phytophagous insects, the larval habitat (host) directly influences offspring development (Burrack *et al.* 2012), and other life history traits, including immunity strength (Chandra 1996; Ojala *et al.* 2005), metabolism (Galvan *et al.* 2008; Kacar *et al.* 2015) and/or physiology (Bellamy *et al.* 2013; Delisle & Bouchard 1995; Gershman *et al.* 2006). It is possible that *D. suzukii* prefers those host plant species whose fruit

provides maximum fitness benefits (Jaenike 1978; Thompson 1998), a relationship which is not unusual among insects and their hosts (Barros & Zucoloto 1999; Craig *et al.* 1989). Often referred to as the “preference-performance” hypothesis or the “Mother knows best” principle (Gripenberg *et al.* 2010), such a relationship with hosts could allow *D. suzukii* to experience higher fitness in novel habitats.

At this time, very little is known about *D. suzukii* habitat choice(s) and the life history consequences that come about from making particular choices. Of the few studies that have investigated host preference, results have been contradictory, possibly owing to differences in experimental design and/or the existence of confounding variables, making it difficult to find the meaning behind the behaviors. For instance, Burrack *et al.* (2012) reported field raspberries to have a much higher infestation rate compared to strawberries, while in a laboratory experimental setting, the opposite was true (Bellamy *et al.* 2013). As well, Abraham *et al.* (2015) and Bellamy *et al.* (2013) found that *D. suzukii* flies were equally attracted to strawberry and raspberry fruits in behavioral and antennal response assays to fruit volatiles. In terms of development, results indicate that larval performance also varies with fruit type, with flies developing on raspberry growing faster and larger compared to flies raised on blueberry, grape, cherry, strawberry and peach (Bellamy *et al.* 2013). However, the underlying reasons for these differences remain unknown.

By not taking into account habitat choice and its influence on life history variation, current and future management strategies may be missing or misinterpreting factors enabling the species to thrive and so may be ill-equipped to dealing with the situation at hand. For instance, farmers might use information on factors influencing population size to plant their crops in configurations that reduce the chances of producing host reservoirs and of *D. suzukii* finding

fitness benefitting hosts. Finally, no matter the mechanism behind choice, information on habitat preference could be used to predict the directional movement of *D. suzukii*, preventing further establishment in present *D. suzukii*-free zones and/or reducing infestation intensity in *D. suzukii*-occupied areas (e.g. planting less preferred hosts as a geographic barrier and deterrent and using preferred hosts as lures).

### ***Meet Your Match(maker):***

#### **The importance of understanding mate choice and its consequences in *D. suzukii***

*Drosophila* use a variety of signals when choosing mates, which may be visual, acoustic, gustatory, tactile and/or olfactory in nature (Greenspan & Ferveur 2000). Often associated with male courtship (Greenspan & Ferveur 2000) and competition for females (Rundle CHCS), secondary sexual traits include the cuticular hydrocarbon profile (CHCs; pheromones involved in courtship, species identity and sex identity; Ferveur 2005), sperm competition (Simmons 2005) and body size (Partridge *et al.* 1987; Pitnick 1991). Among closely related *Drosophila* species (and even between members of the same species), preference for mates is highly variable, with variation in individual fitness strongly tied to the outcome of mate choice (Singh & Singh 2014). In *D. suzukii*, courtship behaviours have recently been described for this species and include signaling via wing movements (Revadi *et al.* 2015) and substrate-borne vibrations (Mazzoni *et al.* 2013). Furthermore, the presence of CHCs produced by *D. suzukii* females greatly stimulates male courtship levels (Revadi *et al.* 2015). The specific expression of these sexual signals may be dependent on the extent of physiological stress experienced by an individual during their development (Buchanan 2000). Specifically, as *Drosophila* sp. raised on different media have been found to display distinct mating patterns (Sharon *et al.* 2010; Najarro *et al.* 2015; Dodd



1989; Abed-Vieillard *et al.* 2016), we may predict that the patterns of courtship and mating outcome may be due to differences in *D. suzukii* rearing environment and be important towards understanding the operation of sexual selection in this species.

There are many ways in which knowledge of how intersexual selection works in *D. suzukii* may be important in predicting fly movement and population size. This is because, as a behavioral motivator, mate choice may lead to flies seeking out desired mates and may act to direct fly movement among and between habitats and fly populations. Besides causing an immediate change in population size through immigration and emigration, such movement may alter population size through the emergence of an indirect fitness effect. In *Drosophila*, the genotypic and phenotypic composition of the social environment has been shown to modulate mating frequency, with higher genetic variability in the social group increasing the number of female matings (Krupp *et al.* 2008). Furthermore, more matings by females has been associated with an increase in female fecundity and offspring genetic variability, both of which could improve individual fitness (Billeter *et al.* 2012). As such, depending on the mating preference of *D. suzukii*, the extent of assortative or disassortative mating of individuals from different populations could influence population size through an increase in the number of offspring produced that also have higher fitness. The act of mate choice itself could have a negative effect on population size/fitness, for instance due to sexually antagonistic traits manifested in offspring (Fedorka & Mousseau 2004) and/or sexual conflict (Pitnick & Garcia-Gonzalez 2002), or have a positive effect on population size, for example through increased offspring fitness (Ala-Honkola *et al.* 2015). Theoretically, *D. suzukii* flies could be more attracted to flies that developed on a particular habitat (host), but because they are locally adapted to their habitat of origin, produce less robust hybrid offspring. In *D. melanogaster*, hybrid inviability has been observed in

populations adapted to media of differing pH (De Oliveira & Cordeiro 1980). And as host availability in North America varies by species and by time of season, it is possible that lineages of flies, having access to one type of fruit for a number of generations, may become locally adapted to a particular fruit type before moving on to the next available host(s), where selective pressures associated with the new habitats may be quite different. For example, in Ontario, cherries and strawberries ripen approximately one month earlier than do raspberries and blueberries (Harvest Ontario 2016). Thus, following the end of a fruiting period, populations of flies will need to switch to a different host crop and if the nutritional composition and quality of the second host differs from the first host, and flies have adapted to produce offspring that develop better on the first host, then performance of the larvae could be negatively affected. Furthermore, as a new generation emerges from the second host, new and different phenotypes may be introduced into the population and, depending on the mate choices of the flies, this could have negative or positive implications for population growth. For instance, in the case where flies prefer to mate with flies of a phenotype that reduces overall female fecundity, then mate choice would have a negative effect on the population growth rate. Consequently, habitat choice can potentially play a significant positive or negative role in individual fitness depending on sexual preferences.

Primarily focusing *D. suzukii* management science on trap design and chemical application has meant that many of the underlying mechanisms of mate choice in this species are largely unknown. By understanding the operation of sexual selection in *D. suzukii*, it may be possible to implement strategies that slow the spread and growth of populations. For instance, farmers could use such information to organize crops in ways that direct *D. suzukii* migration and/or control the

phenotype/genotype of flies that are able to interbreed and so determine the fitness of populations in the present and future.

### **The relationship between mate choice and habitat choice**

Just as mate choice is influenced by habitat choice, habitat choice is likely influenced by mate choice. Distinguishing how each sways the other in choice situations will be important for understanding fly movement and individual fitness in *D. sukii*. For example, flies that developed on one type of habitat may prefer mates with traits that are not attractive to flies that developed on a different habitat. Dodd *et al.* (1989) observed this type of behavioral isolation in populations of fruit flies raised on either a starch- or maltose-based media. In both cases, the fitness of the mating couples and their offspring may also be affected differently for reasons described above. Because of the potential interplay between mate and host choice, understanding the whole picture, which means understanding the relationship between host and mate choice, will be far more effective for management purposes.

### **Objectives and significance**

The choices that organisms make can have large impacts on individual fitness and the fitness of the species as a whole (Hassell & Southwood 1978; Kokko *et al.* 2003). Currently, we understand very little of the choices that *D. sukii* make in the environment and, being a highly successful invasive species that inhabits large expanses of variable landscapes, understanding *D. sukii*'s choices may be of strong importance to their success and, potentially, their management.

In my research, two main themes are investigated. The first theme involves examining the effects of nutrition on adult *D. suzukii* behaviour and larval performance. Using a nutritional geometry framework (Simpson and Raubenheimer 1993), I assess (in Chapter 2), the relationship between *D. suzukii*'s performance and its nutritional acquisition in both choice and no-choice environments. Although nutritional geometry studies have been conducted with various *Drosophila* species, including *D. melanogaster*, none have exploited this formidable tool in studies with *D. suzukii* and, by using such a method, this research is able to identify currently unknown underlying factors that may be influencing *D. suzukii* host-choice preference(s) and life history in different environments.

In the second theme, I study the relationship between habitat choice and mate choice on individual fitness while building off of the geometric framework devised in the first part (Chapter 3). I look at the effect to which the nutritional background of adults (aka the nutritional profile of the diet during development) shapes mating preference in both choice and no-choice situations and, using fecundity and offspring survival as a proxy for fitness, assess the importance of mate choice on an individual scale. Finally, to illustrate the fitness-related relationship between preference and performance, I integrate the observations on preference for hosts, developmental performance and preference for mates based on nutritional background.

## References

- Abed-Vieillard, D., Cortot, J., Everaerts, C. & Ferveur, J-F. 2013. Choice alters *Drosophila* oviposition site preference on menthol. *Biology Open*, **3**: 22-28. doi 10.1242/bio.2013673
- Abraham, J., Zhang, A., Angeli, S., Abubeker, S., Michel, C., Feng, Y. and Rodriguez-Saona, C. 2015. Behavioral and antennal responses of *Drosophila suzukii* (diptera: drosophilidae) to volatiles from fruit extracts. *Environmental Entomologist*, **44**: 356-367.
- Ala-Honkola, O., Laine, L., Pekkala, N., Kotiaho, J., Honkola, T. and Puurtinen, M. 2015. Males benefit from mating with outbred females in *Drosophila littoralis*: male choice for female genetic quality? *International Journal of Behavioral Biology*, **121**: 577-585. doi 10.1111/eth.12369
- Atallah, J., Teixeira L, Salazar, R, Zaragoza, G. & Kopp, A. 2014. The making of a pest: the evolution of a fruit-penetrating ovipositor in *Drosophila suzukii* and related species. *Proceedings of the Royal Society* 281: 20132840.
- Asplen, M.K., Anfora, G., Biondi, A., Choi, D-S, Chu, D., Daane, K.M., Gibert, P., Gutierrez, A.P., Hoelmer, K.A., Hutchison, W.D, Isaacs, R., Jiang, Z-L, Karpati, Z., Kimura, M.T., Pascual, M., Philips, C.R., Plantamp, C., Ponti, L., Vetek, G., Vogt, H., Walton, V.M., Yu, Y., Zappala, L. and Desneux, Nicolas. 2015. Invasion biology of spotted wing *Drosophila* (*Drosophila suzukii*): a global perspective and future priorities. *Journal of Pest Science*, **88**: 469-494. doi 10.1007/s10340-015-0681-z
- Bateman, A.J. 1949. Analysis of data on sexual isolation. *Evolution*, **3**: 174-177.
- Barron, A.B. 2001. The life and death of Hopkins' host-selection principle. *Journal of Insect Behavior*, **14**: 725-737.
- Barros, H.C.H. & Zucoloto, F.S. 1999. Performance and host preference of *Ascia monuste* (Lepidoptera, Pieridae). *Journal of Insect Physiology*, **45**: 7-14.
- Bellamy, D.E., Sisterson, M.S. & Walse, S.S. 2013. Quantifying host potentials: Indexing postharvest fresh fruits for Spotted Wing *Drosophila*, *Drosophila suzukii*. *PLOS ONE*, **8**(4): e61227.
- Billeter, J-C, Samyukta, J., Stepek, N, Azanchi, R. and Levine, J.D. 2012. *Drosophila melanogaster* females change mating behaviour and offspring production based on social context. *Proceedings of the Royal Society of London B*, **279**: 2417-2425. doi 10.1098/rspb.2011.2676
- Bolda, M. P., Goodhue, R.E. and Zalom, F.G. 2010. Spotted Wing *Drosophila*: potential economic impact of a newly established pest. Agricultural resource economics. Update, University of California Giannini. *Foundation of Agricultural Economics*, **13**: 5- 8.

Buchanan, K. L. 2000 Stress and the evolution of condition-dependent signals. *Trends in Ecology & Evolution* 15: 156-160

Burrack, H.J., Fernandez, G.E., Spivey, T. & Kraus D.A. 2012. Variation in selection and utilization of host crops in the field and laboratory by *Drosophila suzukii* matsumara (diptera: drosophilidae), an invasive frugivore. *Pest Management Science*, **69**: 1173-1180. doi 10.1002/ps.3489

Chandra, R.K. 1996. Nutrition, immunity and infection: from basic knowledge of dietary manipulation of immune responses to practical application of ameliorating suffering and improving survival. *Procedures of the National Academy of Science USA*, **93**:14304–14307.

Cini, A., Ioraitti, C. & Anfora, G. 2012. A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. *Bulletin Insectology* **65**:149–160.

Coyne, J.A. and Milstead, B. 1987. Long-distance migration of *Drosophila*. 3. Dispersal of *D. melanogaster* orchard. *The American Naturalist*, **130**(1):70-82.

Craig, T.P., Itami, J.K. & Price, P.W. 1989. A strong relationship between oviposition preference and larval performance in a shoot-galling sawfly. *Ecology*, **70**(6): 1691-1699.

Davis, R.S., Alston, D and Corey, V. *Spotted Wing Drosophila*. [online]. Utah pests fact sheet, university of utah cooperative extension, ent-140-10. available: <http://extension.usu.edu/files/publications/publication/ent-140-10.pdf> [15 april 2011].

Davis, J.M. and Stamps, J.A. 2004. The effect of natal experience on habitat preferences. *TRENDS in Ecology and Evolution*, **19**(8): 411-416.

Delisle, J. & Bouchard, A. 1995. Male larval nutrition in *Choristoneura rosaceana* (lepidoptera: tortricidae): an important factor in reproductive success. *Oecologia*, **104**: 508-517.

De Oliveira, A.K., Cordeiroa, R. 1980. Adaptation of *drosophila-willistonie* experimental populations to extreme ph medium. *Heredity*, **44**: 123-130.

Dodd, D.M.B. 1989. Reproductive isolation as a consequence of adaptive divergence in *Drosophila pseudoobscura*. *Evolution*, **43**(6): 1308-1311.

Fedorka, K.M. and Mousseau, T.A. 2004. Female mating bias results in conflicting sex-specific offspring fitness. *Nature*, **429**: 65-67.

Ferguson, C.T.J., O'Neill, T.L., and Elwyn Isaac, R. The sexually dimorphic behaviour of adult *Drosophila suzukii*: elevated female locomotor activity and loss of siesta is a post-mating response. *Journal of Experimental Biology*, **218**: 3855-3861

Ferveur, J-F. 2005. Cuticular hydrocarbons: Their evolution and roles in *Drosophila* pheromonal communication. *Behavior Genetics*, **35**(3):279-295

Galvan, T.L., Koch, R.L., & Hutchison, W.D. 2008. Impact of fruit feeding on overwintering survival of the multicolored Asian lady beetle, and the ability of this insect and paper wasps to injure wine grape berries. *Entomologia Experimentalis et Applicata*, **128**: 429-436.

Gershman, B., Puig, O., Hang, L., Peitzsch, R.M., Tatar, M. & Garofalo, R.S. 2006. High resolution dynamics of the transcriptional response to nutrition in *Drosophila*: a key role for dfoxo. *Physiological Genomics* **29**: 24–34.

Hamilton, K.. *Wisconsin pest bulletin*. [online]. available: <https://datcpservices.wisconsin.gov/pb/pests.jsp?categoryid=32&issueid=155> [15 april 2011].

Greenspan, R.J., & Ferveur, J.F. 2000. Courtship in *drosophila*. *Annual Reviews Genetics*, **34**:205–232.

Harvest Ontario. 2016. Fresh fruit & vegetable availability guide. <http://www.harvestontario.com/seasonal.php>

Harris, D.W., Hamby, K.A., Wilson, H.E. and Zalom, F.G. 2014. Seasonal monitoring of *Drosophila suzukii* (Diptera: Drosophilidae) in a mixed fruit production system. *Journal of Asia-Pacific Entomology*, **17**: 857-864.

Hassell, M.P. and Southwood, T.R.E. 1978. Foraging strategies of insects. *Annual review of ecology and systematics*, **9**: 75-98.

Heimpel, G.E, Frelich, L.E, Landis, D.A., Hopper, K.R, Hoelmer, K.A., Sezen, Z., Asplen, M.K. and Wu, K. 2010. European buckthorn and asian soybean aphid as components of an extensive invasional meltdown in North America. *Biological Invasions*, **12**:2913–2931

Iglesias, L.E., Nyoike, T.W. & Liburd, O.E. 2014. Effect of trap design, bait type, and age on captures of *drosophila suzukii* (diptera:drosophilidae) in berry crops. *Horticultural Entomology*, **107**: 1508-1518.

Isaacs, R., Hahn, N., Tritten, B. and Garcia, C. *Spotted Wing Drosophila – a new invasive pest of Michigan fruit crops*. [online]. msuextension bulletin e-3140 (2010). available: <http://www.ipm.msu.edu/swd/e-3140.pdf> [15 april 2011].

Jaenike, J. 1978. On optimal oviposition behaviour in phytophagous insects. *Theoretical Population Biology*, **14**: 350-356.

Jaenike, J. 1990. Host specialization in phytophagous insects. *Annual Review of Ecology and Systematics*, **21**: 243-273.

Jagadeeshan, S., Shah, U., Chakrabarti, D. and Singh, R.S. 2015. Female choice or male sex drive? The advantages of male body size during mating in *Drosophila melanogaster*. *PLoS ONE* **10**(12). doi 10.1371/journal.pone.0144672

Jones, J.S, Bryant, S.H., Lewontin, R.C., Moore, J.A. and Prout, T. 1981. Gene flow and the geographical distribution of a molecular polymorphism in *Drosophila pseudoobscura*. *Genetics*, **98**: 157-178.

Kacar, G., Wang, X., Stewart, T.J. & Daane, K.M. 2015. Overwintering survival of *drosophila suzukii* (diptera: drosophilidae) and the effect of food on adult survival in california's san joaquin valley. *Environmental Entomology*, **0**: 1-9. doi 10.1093/ee/nvv182

Kaneshiro, K.Y. 1983. *Drosophila* (sophophora) *suzukii* (matsumura). *Proceedings of the Hawaiian Entomology Society*, **24**: 179.

Kanzawa, T. 1936. Studies on *drosophila suzukii* mats. *Journal of Plant Protection*, **23**, 66–70.

Kokko, H., Brooks, R., Jennions, M.D. and Morley, J. 2003. The evolution of mate choice and mating biases. *Biological Sciences*, **270**: 653-654.

Lee, J.C., Bruck, D.J., Curry, H., Edwards, D., Haviland, D.R., Steenwyk, R.A.Y., Yorgey, B.M. 2011. The susceptibility of small fruits and cherries to the spotted-wing drosophila, *Drosophila suzukii*. *Pest Management*, **67**: 1358-1367.

LeFranc, A. and Bundgaard. 2000. The influence of male and female body size on copulation duration and fecundity in *Drosophila melanogaster*. *Hereditas*, **132**: 243-247.

Krupp, J.J., Kent, C., Billeter, J-C., Azanchi, R., So, A.K.-C., Schonfeld, J.A., Smith, B.P., Lucas, C. and Levine, J.D. 2008. Social experience modifies pheromone expression and mating behavior in male *Drosophila melanogaster*. *Current Biology*, **18**: 1373-1383.

Lee, J.C., Shearer, P.W., Barrantes, L.D., Beers, E.H., Burrack, H.J., Dalton, D.T., Dreves, A.J., Gut, L.J., Hamby, K.A., Haviland, D.R., Isaacs, R., Nielsen, A.L., Richardson, T., Rodriguez-saona, C.R., Stanley, C.A., Walsh, D.B., Walton, V.M., Yee, W.L., Zalom, F.G. & Bruck, D.J. 2013. Trap designs for monitoring *Drosophila suzukii* (diptera: drosophilidae). *Environmental Entomology*, **42**: 1348-1355.

Lin, Q-C., Zhai, Y-F., Zhou, C-G., Li, L-L., Zhuang, Q-Y., Zhang, X-Y., Zalom, F.G. & Yu, Y. 2014. Behavioral rhythms of *Drosophila suzukii* and *Drosophila melanogaster*. *Florida Entomologist*, **97**: 1424-1433.

Malogolowkin-Cohen, C.H., Solima Simmons, A. and Levene, H. 1965. A study of sexual isolation between certain strains of *Drosophila paulistorum*. *Evolution*, **19**:95-103.



- Mazzoni, V., Gianfranco, A., Virant-Doberlet, M. 2013. Substrate vibrations during courtship in three *Drosophila* species. *PLOS ONE*, **8**.
- Merrell, D.J. 1950. Measurement of sexual isolation and selective mating. *Evolution*, **4**:326-331.
- Najarro, M.A., Sumethasorn, M., Lamoureux, A. & Turner, T.L. 2015. Choosing mates based on the diet of your ancestors: replication of non-genetic assortative mating in *Drosophila melanogaster*. *Peer Journal*, **3**.
- Ojala, K., Julkunen-tiitto, R., Lindstrom, L. & Mappes j. 2005. Diet affects the immune defence and life-history traits of an arctiid moth *Parasemia plantaginis*. *Evolutionary Ecology Research*, **7**: 1153-1170.
- Partridge, L., Green, A. & Fowler, K. 1987. Effects of egg production and of exposure to males on female survival in *Drosophila melanogaster*. *Journal of Insect Physiology*, **33** (10): 745-749.
- Pitnick, S. 1991. Male size influences mate fecundity and remating interval in *Drosophila melanogaster*. *Animal Behavior*, **41**: 735-745.
- Pitnick, S. and Garcia-Gonzalez. 2002. Harm to females increases with male body size in *Drosophila melanogaster*. *Proceedings of the Royal Society of London B*, **269**: 1821-1828. doi 10.1098/rspb.2002.2090
- Pizzari, T. and Gardner, A. 2012. The sociobiology of sex: inclusive fitness consequences of inter-sexual interactions. *Biological Sciences*, **367**(1600): 2314-2323.
- Revadi, S., Lebreton, S., Witzgall, P., Anfora, G., Dekker, T. & Becher, P.G., 2015. Sexual behavior of *Drosophila suzukii*. *Insects*, **6**: 183-196 doi 10.3390/insects6010183
- Saguez, J., Lasnier, J. & Vincent, C. 2013. First record of *Drosophila suzukii* in Quebec vineyards. *Journal International des Sciences de la Vigne et du Vin*, **47**: 69-72.
- Sharon, G., Segal, D., Ringo, J.M., Hefetz, A., Zilber-rosenberg, I., Rosenberg, E. & Collier, R.J. 2010. Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America*, **107**: 20051-20056.
- Simmons, L.W. 2005. The evolution of polyandry: sperm competition, sperm selection and offspring viability. *Annual Reviews of Ecology, Evolution & Systematics*, **36**: 125-46. doi10.1146/annurev.ecolsys.36.102403.112501
- Singh, A. and Singh, B. 2014. Role of sexual selection in speciation in *Drosophila*. *Genetica*, **142**: 23-41. doi 10.1007/s1079-013-9751-4

Snook, R.R., Chapman, T., Moore, P.J., Wedell, N. and Crudgington, H.S. 2009. Interactions between the sexes: new perspectives on sexual selection and reproductive isolation. *Evolutionary Ecology*, **23**: 71-91. doi 10.1007/s10682-007-9215-3

Stalker, H.D. 1942. Sexual isolation studies in the species complex *Drosophila virilis*. *Genetics*, **27**:238-257.

Swoboda-Bhattarai, K.A. and Burrack, H.J. 2014. Influence of edible fruit coatings on *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) oviposition and development. *International Journal of Pest Management*, **60**(4): 279-286. <http://dx.doi.org/10.1080/09670874.2014.971453>

Thompson, J.N. 1998. Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomologia Experimentalis et Applied*, **47**: 3-14.

Timmeren, S.T., Isaacs R. 2013. Control of spotted wing drosophila, *Drosophila suzukii*, by specific insecticides and by conventional and organic crop protection programs. *Crop Protection* **54**: 126-133.

Walsh, D.B., Bolda, M.P., Goodhue, R.E., Dreves, A.J., Lee, J., Bruck, D.J., Walton, Y.M., O'neal, S.D. & Zalom, F.G. 2011. *Drosophila suzukii* (diptera: drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential. *Journal of Integrated Pest Management*, **2**. doi 10.1603/ipm10010

## **CHAPTER 2**

### NUTRITIONAL GEOMETRY AND FITNESS CONSEQUENCES IN THE SPOTTED-WING FRUIT FLY, *DROSOPHILA SUZUKII*

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## **Preamble**

The following chapter was written as a manuscript in the style of *The Journal of Ecology and Evolution*, where it has been submitted for consideration.

## **Abstract**

Since its arrival to North America less than a decade ago, the invasive Spotted-Wing fruit fly (*Drosophila suzukii*), has inflicted substantial economic losses to the soft-fruit agriculture industry due to its ability to oviposit into ripening fruits. There is an urgent need for more effective management approaches for this species, but little is known about the factors influencing the behavioural choices made by *D. suzukii* when selecting hosts, or the consequences experienced by their offspring developing in different environments. Using a nutritional geometry methodology, we found that the ratio of carbohydrates-to-protein (P:C) present in media greatly influenced adult *D. suzukii* behavior and offspring development. Whereas for oviposition and association behaviours, carbohydrate-rich foods were preferred by flies, we also saw that larval survival and eclosion rate were strongly dependent on protein availability. Here, we explore the preference performance hypothesis (PPH), in which females are predicted to oviposit on medias that provide the greatest offspring benefits, in regards to its relevance in *D. suzukii* behavior and consequences for management. Our results provide valuable insight into the ecology and evolution of this species that may lead to more effective management strategies.

## Introduction

The spotted-wing fruit fly, *Drosophila suzukii*, is an invasive agricultural pest known to attack a number of soft-fruit species including blueberries, blackberries, strawberries, raspberries and occasionally grapes (Bellamy *et al.* 2013; Lee *et al.* 2011). Unlike most other drosophilids which seek out rotting fruit, *D. suzukii* exploits a different environmental niche; ripe or ripening fruit (Walsh *et al.* 2011). This is facilitated by the females' large, serrated, ovipositor which is used to cut the fruit's skin before laying eggs directly into the mesocarp (Atallah *et al.* 2014), ultimately leaving it unmarketable (Walsh *et al.* 2011). Since its initial arrival in North America, this species has resulted in yield losses of up to 80% (USDA NASS 2009) with Bolda *et al.* (2010) estimating an annual economic loss of \$500 million USD in California, Oregon and Washington alone. In order to control and/or manage this species, it is imperative that we understand the factors that contribute to its success. It is thus, somewhat surprising that there has been relatively little research conducted on the foraging behavior in this species or on the relationship between host-choice and life history traits, and what studies have been conducted have been of limited scope, have not controlled for potentially confounding variables, and/or have not explored the underlying reasons for any differences in behaviour or performance. Such information on host preferences and offspring performance is of great value for modeling population growth, monitoring spread and for designing better *D. suzukii* management plans.

To date, there is very limited information on the nature of host preferences in *D. suzukii* and in those that have been conducted, extrapolating the meaning behind the results is made difficult by confounding variables and/or non-rigorous methodology. Using a series of olfactory choice experiments, Abraham *et al.* (2015) found that flies were more attracted to the volatile scents originating from raspberries and strawberries than they were to scents obtained from

cherries and blueberries, while Bellamy *et al.* (2013) found that the scents of raspberries, strawberries and blackberries were more attractive to the volatiles emanating from peaches, cherries, blueberries and grapes. These results are suggestive that *D. suzukii* does exhibit host-preferences but do not provide insight on actual egg-laying rates, or subsequent offspring performance. Field studies conducted by Burrack *et al.* (2013) found that infestation rates varied considerably between crop species and varieties, and that infestation rates differed considerably between years and often depended on the type of plot in which fruits were grown. In the lab, simple (no-choice or single-choice) oviposition preference assays using fruit, revealed that flies laid more eggs in raspberries than in blackberries, strawberries or blueberries (Burrack *et al.* 2013). In contrast, in Abraham *et al.* (2015)'s assays—using pureed fruit media— females laid far more eggs in strawberry media than all other choices. However as fruit type/varieties differ in numerous ways, including colour, texture, size, shape & phenology, that could influence oviposition rates, as indicated by a complementary assay which revealed that surface hardness (and thus penetration force) dramatically influenced the number of eggs laid (Burrack *et al.* 2013), which could obscure potential host preferences based on (for instance) differences in fruit chemical composition. The survivorship and development of offspring also appears to depend on the host chosen, as Bellamy *et al.* (2013) reported that larvae developed faster and grew larger on raspberries than on blueberries, grapes, cherries, strawberries and peaches, but the reason(s) for these differences is unknown. In our study, we set out to examine a potentially important underlying variable (the protein-to-carbohydrate ratio) that may be responsible for differences in host preference, an important first step towards understanding the ecology and life-history of this invasive species.

For many insect species, proteins and carbohydrates are the two macronutrients that are most important for growth, reproduction and survival (Andersen *et al.* 2009; Carrel & Tanner 2002; Jensen *et al.* 2015; Lihoreau *et al.* 2016; Maklakov *et al.* 2008; May *et al.* 2015; Morimoto & Wigby 2016; Raubenheimer & Simpson 2003; Rodrigues *et al.* 2016). While dietary protein is an important aspect for the stimulation of oogenesis and regulating vitellogenesis in females and for stimulating the production of sperm in males (Fenson *et al.* 2009; Fenson & Taylor 2012; Jensen *et al.* 2015; Lee *et al.* 2008; Lihoreau *et al.* 2016; Maklakov *et al.* 2008; Pirk *et al.* 2010; Reddiex *et al.* 2013), carbohydrates are used principally as sources of energy for fat and glycogen synthesis (Friend 1958). Carbohydrates, being the more easily metabolized of the two (Maklakov *et al.* 2008; South *et al.* 2011), may be the more important nutritive resource for species that engage in high-energy activities such as flying. Among the fruit varieties parasitized by *D. suzukii*, the composition of proteins and carbohydrates vary in ratio (P:C) and concentration (P+C), and may be underlying factor(s) influencing host preference, offspring survival and egg-to-adult development in this species. For instance, raspberries, strawberries and blueberries, have P:C ratios of 1:5, 1:7 and 1:15, and P+C concentrations (g/kg) of 49, 56 and 108, respectively (Table S1). Both protein and carbohydrate availability have been shown to be strongly associated with variation in foraging behavior, oviposition rate, development, reproduction, and longevity in a number of insect species (Jensen *et al.* 2015; Lee *et al.* 2007; Lihoreau *et al.* 2016; Maklakov *et al.* 2008; Morimoto & Wigby 2016; Reddiex *et al.* 2013; Rodrigues *et al.* 2015 ). For instance, while hissing cockroach, *Gromphadorhina portentosa*, females prefer to feed on relatively high-protein foods (Carrel & Tanner 2002), female *D. melanogaster* seem to prefer to feed on foods with relatively higher carbohydrates, as sites for both feeding and egg-laying (Lihoreau *et al.* 2016; Rodrigues *et al.* 2016). Such dietary



preferences may be the result of differences in the energy accessibility between the two macronutrients. Female choice for oviposition is also important for offspring success in a number of species (e.g. Andersen *et al.* 2010; Morimoto & Wigby 2016; Rodrigues *et al.* 2015), since the characteristics of the natal diet during development influences numerous traits correlated with fecundity and mating success. One such trait is adult body size, with larger males and females emerging from media of greater nutritional quality (e.g. Thomas 1993). Large individuals have been known to exhibit higher fecundity (Kaspi *et al.* 2002; LeFranc & Bundgaard 2000; Tantaway 1960) and mating success (Partridge *et al.* 1987ab). Often referred to as the “preference-performance” hypothesis or the “Mother knows best” principle (Gripenberg *et al.* 2010), it argues that selection will act on a species such that females will evolve a preference to oviposit in environments that will maximize their offspring’s success. While this hypothesis has found some empirical support (Bellamy *et al.* 2013; Hanks *et al.* 1993; Rausher 1979), there are several documented cases of species in which females deposit eggs in suboptimal conditions indicating the preference-performance relationship is not a ubiquitous phenomenon, even among closely related taxa (Konig *et al.* 2016; Lihoreau *et al.* 2016; Rausher 1979). Preliminary evidence suggests that *D. suzukii* might be preferentially ovipositing in fruit types that maximize fitness, as females may lay more eggs in fruit type(s) that result in higher offspring survival (Bellamy *et al.* 2013; Burrack *et al.* 2013). However, because of the number of potentially confounding variables that accompany the use of fruit in such studies (including differences in penetration force), more standardized studies are needed to test this hypothesis.

While *D. suzukii* are known to parasitize a number of different farmed and wild fruit species in North America –which vary widely in their protein to carbohydrate ratios– there has yet to be (to the best of our knowledge) any systematic examination of the nutritional geometry

of their feeding/egg-laying behavior, or a quantification of offspring performance in hosts differing in their protein-to-carbohydrate ratios. This is an important area of research with numerous implications for the study of this species. While *D. melanogaster* females may seek out medias that are relatively abundant in carbohydrates (Lihoreau *et al.* 2016; Rodrigues *et al.* 2016), the same may not necessarily be the case for *D. suzukii*, as the former develop in rotting fruit, which are colonized by protein-rich molds and yeasts (Begon 1986; Da Cunha *et al.* 1951; Cooper 1959), while the latter develop in unspoiled fruits where (at least initially) the proteins available from the microbial community will be limited, and may primarily come from the fruit itself. As a result, there may be considerable differences in the selection experienced by these two species, which has led to the evolution of different sets of adaptations. A recent study by Jaramillo *et al.* (2015) examined numerous life-history traits in larvae grown on blueberries (P:C ratio of ~1:15) and on a standard laboratory fly media (P:C ratio of ~1:3). Surprisingly, they found that while development times differed between the two groups, there was no significant difference between the blueberry and yeast-reared *D. suzukii* for most variables measured (including survivorship, body size, rate of ovarian maturation, and fecundity over the first 14 days of life). While Jaramillo *et al.* (2015)'s results are suggestive that *D. suzukii* has evolved the capacity to survive on relatively little dietary protein, because this study did not explore the entire spectrum of P:C ratio diets that flies may encounter in the wild, much remains unknown about the nature of the relationship between larval diet and development in this species.

Our assays are based upon the concept of nutritional geometry, which was developed by Simpson and Raubenheimer (1993) to dissect the relationship between an organism's performance and their nutritional acquisition by manipulating two variables (in our case, protein and carbohydrates) across a "landscape". To assess the importance of P:C ratio on *D. suzukii*

foraging and egg-laying behavior, we measured the activity and oviposition preference of flies among eight different artificial medias in ‘cafeteria’-style arenas. To complement this study, we also tested the consistency of female behaviors by measuring egg-laying preference in a no-choice experiment using the eight different diets. Finally, we addressed the importance of the natal diet on development and adult traits by comparing the development (egg-to-adult survival, eclosion rates and adult weights) of larvae under standardized competitive conditions on each of eight experimental diets. The ultimate goal of this study was to determine the importance of differences in protein to carbohydrate ratios in diets on adult foraging behavior and life history traits in *D. suzukii*, information that is potentially useful in furthering our understanding of evolutionary life-history traits as well as for managing and/or mediating the effects of this invasive pest species.

## **Materials & Methods**

### ***Drosophila suzukii* Population History & Culture Protocols**

All flies used in this experiment originate from a large (~1400 adults/generation) laboratory population of *Drosophila suzukii*. This population was founded from a sample of individuals isolated from blackberries and raspberries collected from a Southern Ontario commercial farm during the summer of 2012 (described in Renkema *et al.* 2016) and which was shared with our lab in 2014 by Dr. Justin Renkema (University of Guelph). Since then, the flies have been cultured under standard laboratory conditions (25°C, 60% humidity, LD 12:12) on Rose’s fly media (that consists of a protein to carbohydrate ratio of ~1:3; Rose 1984). The population is cultured on a 21 day cycle, whereby on day 1 of the cycle, flies are mixed *en masse* under light anesthesia (CO<sub>2</sub>) and transferred to a fresh set of vials containing ~10 ml of fly media, with 20-

25 flies per vial. After 48 hours in these vials, the flies are transferred to a second set of fresh vials before being discarded 48 hours later.

### ***Experimental Diets***

Preference and developmental performance of *D. suzukii* was analyzed using eight artificial diets in which the protein to carbohydrate ratio (P:C) was experimentally manipulated (P:C 1:12, 1:6, 1:3, 1:1, 2:1, 4:1, 8:1, 24:1). We chose these P:C ratios based on two criteria. First, these ratios allow us to compare our results with the work of other researchers that use similar nutritional geometry methods to measure *Drosophila* behavior and life history, specifically those studies by Lihoreau *et al.* (2016) and Rodriguez *et al.* (2015). Second, these values span the range of potential P:C ratios encountered by *D. suzukii* in their natural habitat. For instance, the ratios 1:12, 1:6, 1:3 and 1:1 span the P:C range commonly observed in farmed and wild fresh fruit commonly attacked by *D. suzukii* in North America (Table S1). The ratio 1:12 resembles the P:C ratio found in floral nectar (Kevan & Baker 1983), a hypothesized energy source for adult *D. suzukii* (Tochen *et al.* 2016). The ratios 2:1, 4:1, 8:1 and 24:1 represent the higher protein content potentially found in rotting fruits (Janzen 1977), a resource that has not yet been tested, to the best of our knowledge, in any oviposition or behavioral studies of *D. suzukii*. While the protein and carbohydrate (P+C) concentrations in fruit vary from 40 to over 200 g/L (Supplementary, Table 1), we chose to focus on a single concentration of 70 g/L, because it represents the average of a large majority of soft fruit species attacked by *D. suzukii* (e.g. raspberries, blackberries and cherries; Table S1) and is similar to the P+C concentration of the standard media (64 g/L) we use to culture our lab population (Rose 1984).

To generate the eight different media, we manipulated the quantities of protein and carbohydrates (Table S2) in each recipe while keeping all other ingredients in the media

constant, as outlined by Lihoreau *et al.* (2016). The ingredients used in our media were very similar to Lihoreau *et al.*'s media, with the exception that we used a 1:1 mix of light and dark corn syrup as the carbohydrate source, instead of sucrose. Corn syrup was used because it contains a 1:1 ratio of fructose and glucose similar to that of fruit, it is the main carbohydrate source we use to culture our lab population and (unlike sucrose) has not been linked to a decrease in female fecundity and lifespan in *Drosophila* (Begon 1986; Hassett 1948; Lushchak *et al.* 2013). We used a 50:50 mix of whey (GNC #386306) and casein (Sigma-Aldrich, C3400) for the protein. All media includes Vanderzant vitamin mixture (Sigma-Aldrich, V1007; 0.25 g/L), methyl paraben (Bioshop, HYD202; 4 g/L) and propionic acid (Fisher Scientific A258-500; 1.5 g/L). In all cases, we added nutritional yeast (10g/L), a common ingredient in the media of similar experiments with *Drosophila* (Lihoreau *et al.* 2016; Rodriguez *et al.* 2015). As such, the protein (0.46g/g) and carbohydrate (0.38g/g) content provided by the yeast was incorporated into the calculations. All media contained 2% agar (Bio Basic Canada Inc. FB0012) and was dyed with green food colouring for greater contrast during egg counting.

### ***Assay 1: Fly Movement and Oviposition in a 'Cafeteria' Choice Environment***

We first set out to quantify the behavior of *D. suzukii* in an environment where they have access to a wide range of P:C media types. We did so by first collecting 160 sets of 15 adult male and 15 female flies from our stock population. These flies were collected on days 18-24 of their culture cycle and were fully mature and likely non-virgin. Each set of flies was placed, under light anesthesia (CO<sub>2</sub>), into a single vial containing 10 ml of lightly-yeasted culture media and stored in an incubator for 48 hours prior to the start of the assay.

The “cafeteria-style” choice arenas (Figure S2.1) we used to measure fly behavior consisted of transparent plastic boxes (KIS Omni Box, 20.3 x 15.9 x 9.6 cm) to which we added

mesh-covered vent holes along the upper edges. At the bottom of each chamber we arranged 8 petri dishes (BD Falcon, 31 mm) that each contained 8 ml of a different P:C media. We placed the dishes in the arenas ~2 hours before the introduction of the flies. The arenas, 80 in total, were housed in a well-lit and quiet room. The orientation of the arenas was alternated in order to account for any spatial effects that may have been present in the room.

The assay began when we transferred (without anaesthesia) two vials of flies (60 flies in total) into each of the arenas. The flies were then left in the chambers for 25 hours, with a survey of fly locations made at 1, 4, 8, 21, 23 and 25 hours post-introduction. This time-range is meant to capture a wide ‘view’ of the potentially variable periods of *D. suzukii* activity levels, as activity levels in this species is known to vary significantly depending on the time of day (Ferguson *et al.* 2015). During each survey, the number and sex of all the flies located on the media surface of each petri dish was recorded. At the end of the 25 hour period, all of the flies were removed from the arenas and the eggs laid on the surface of each of the media in the petri dishes were immediately counted.

### ***Assay 2: Oviposition in a No-Choice Scenario***

In order to investigate egg laying behavior in a “no-choice” environment, we collected 880 females from our lab population. These flies were collected on days 18-24 of their culture cycle, were fully mature, and presumably mated. Each female was placed, individually, into a vial containing 2 ml of one of the 8 P:C medias described above. Vials with flies were incubated for 36 hours, before all females were removed and the number of eggs laid in each vial was counted.

### ***Assay 3: Larval Development on the Eight Different P:C Diets***

To quantify the development of *D. suzukii* larvae on media with different P:C ratios, (but standardized initial levels of larval competition), we collected eggs laid by adult flies from our

lab population. This was done by placing flies into half-pint laying chambers outfitted with 35 mm petri dish lids (BD Falcon) containing a grape-agar media (Sullivan *et al.* 2000) for ~18 hours. Eggs were sorted into groups of 20 and transferred into vials containing 10 ml of one of the 8 P:C media types (50 replicates per treatment). These vials were incubated, and starting 12 days later, all eclosed adult flies were removed, sexed and counted every 48 hours, a schedule that continued until day 22. The first 50 females and the first 50 males collected from each media treatment on the census days were immediately frozen for later weighing. Flies were weighed by first placing them into a drying oven set at 70°C overnight and weighed on a Sartorius Ultramicrobalance to the closest 0.1 µg.

Later, to investigate the possibility that *D. suzukii* larvae might benefit from the protein originating from microbial growth in the media, as is seen in other *Drosophila* species (Begon 1986; Da Cunha *et al.* 1951; Cooper 1959; Lihoreau *et al.* 2016), we conducted a follow-up experiment. In this assay, we omitted the addition of antimicrobials (Tegosept and propionic acid) in the media, but otherwise followed the same experimental protocols used in the first developmental assay, except with fewer replicates (25) per treatment. Flies that eclosed as adults were removed, sexed and counted every 24 hours for a total of 22 days.

### ***Statistical Analysis***

We used R 3.3.1 (R Core Team 2016) for all statistical analyses. The location of male and female flies on the eight different P:C medias was analyzed both together, and separately by sex using general linear models (GLMs) constructed with quasi-binomial error distributions. In each model the sum of all counts of flies on the surface of the petri dishes containing media over the course of the 25 hour observation period was the dependent variable and the total count of flies on all petri dishes in the chamber throughout the assay was the binomial denominator. The significance

of treatment was determined using the *Anova* function (in the *car* package), with type II sums of squares. To determine the differences in egg-laying behaviour associated with different media types we constructed a GLM with a quasipoisson error distribution. A model was created for each class of behavioural or fitness response, with treatment as an independent factor. The significance of treatment was determined using the *Anova* function, and specific differences in the number of eggs laid on each media type was determined using a Tukey HSD test. Egg-laying activity in the no-choice scenario was also analyzed using GLM with a quasipoisson error distribution. To see if females exhibited a similar preference for egg-laying site when given no choice versus a choice in media, we performed a Spearman correlation test in which we examined the number of eggs laid on each type of media where flies were given a choice and no choice. Survivorship among the different treatments was analyzed by fitting a GLM with a quasibinomial logit to the number of flies that eclosed in each vial in each treatment as the dependent variable and the initial number of eggs added to the vial was the binomial denominator. In order to measure potential differences in eclosion rate in different media we performed a Kruskal-Wallis (rank-sum) test on the number of flies that eclosed each day followed with a *post-hoc* comparisons of medians using the *kruskal.mc* function in the *pgirmess* package. Finally, the normally distributed male and female fly weights were analyzed separately by sex using an one-way Analysis of Variance (ANOVA) with *post-hoc* Tukey HSD tests to determine where the differences in adult weight lay between media treatments.

## **Results**

### ***Fly Distribution & Oviposition in “Choice” Chambers***



Adult *D. suzukii* flies distributed themselves non-randomly among the eight P:C media in the choice arenas, with the greatest number of flies associating on the highest carbohydrate (1:12) media over the 25 hours of observation. This pattern was seen in both sexes when they were analyzed separately by sex (GLM: females: LLR  $\chi^2=350.05$ ,  $df=7$ ,  $p<0.001$ ; males: LLR  $\chi^2=610.18$ ,  $df=7$ ,  $p<0.001$ ) and when pooled together (LLR  $\chi^2=750.11$ ,  $df=7$ ,  $p<0.001$ ; Figure S2.2). Similarly, the number of eggs laid on the media differed significantly between media types (GLM, LLR,  $\chi^2=1458.9$ ,  $df=7$ ,  $<0.001$ ). The greatest number of eggs were laid in the media with highest carbohydrate to protein ratio, 1:12 (mean =  $42.7 \pm 2.98$  eggs or  $\approx 40\%$  of eggs laid/chamber) and progressively fewer eggs were laid on media with a decreasing carbohydrate to protein ratio (Figure 2.1). When the C:P and P:C ratio was treated as a continuous variable, we saw a significant positive relationship and negative relationship with oviposition rate, respectively (Figure S2.5, ANOVA  $F=2289.5$ ,  $df=1$ , 638,  $p<2.2 \times 10^{-16}$ ; ANOVA  $F=175.37$ ,  $df=1$ , 638,  $p<2.2 \times 10^{-16}$ ).

### ***Oviposition in “No-choice” Vials***

The median number of eggs laid by single *D. suzukii* in the “no-choice” vials differed between the media treatments (GLM, LLR,  $\chi^2=45.64$ ,  $df=7$ ,  $<0.001$ ). We saw greater oviposition on those media with low P:C ratios (such as 1:12 and 1:6) than on those with high ratios (i.e. 24:1). When the C:P and P:C ratio was treated as a continuous variable, we saw a significant positive relationship and negative relationship with oviposition rate, respectively (Figure S2.6, ANOVA  $F=21.135$ ,  $df=1$ , 878,  $p<4.9 \times 10^{-6}$ ; ANOVA  $F=25.545$ ,  $df=1$ , 878,  $p<5.3 \times 10^{-7}$ ). We observed a significant positive correlation between the number of eggs laid on the media types in the no-choice and choice experiments (Spearman’s  $Rho=0.785$ ,  $S = 18$ ,  $df=7$ ,  $p = 0.028$ ).

### ***Development & Survivorship***

The egg-to-adult survivorship of *D. suzukii* differed depending on the type of media that the larvae developed on, albeit with opposing trends between the two experiments. In media with antimicrobials added (T+; GLM: LLR  $\chi^2=557.15$ ,  $df=7$ ,  $p<0.001$ ), the greatest mortality arose on carbohydrate-rich medias, and the greatest survivorship on protein-rich medias (Figure 2.2A), whereas, in the media in which antimicrobials were omitted (T-; GLM: LLR  $\chi^2=46.54$ ,  $df=7$ ,  $p<0.001$ ), the greatest mortality arose on protein-rich medias, and the greatest survivorship on carbohydrate-rich media (Figure 2.2B). When the C:P and P:C ratio was treated as a continuous variable, we saw a significant positive relationship and negative relationship with survivorship in both development experiments, respectively (Figure S2.7, with antimicrobials: ANOVA  $F=539.61$ ,  $df=1$ ,  $396$ ,  $p<2.2\times 10^{-16}$ ; ANOVA  $F=76.524$ ,  $df=1$ ,  $396$ ,  $p<2.2\times 10^{-16}$ ; without antimicrobials: ANOVA  $F=5.065$ ,  $df=1$ ,  $172$ ,  $p=0.026$ ; ANOVA  $F=36.795$ ,  $df=1$ ,  $172$ ,  $p<8.1\times 10^{-9}$ ). The number of males and females that eclosed did not differ between the eight media types, indicating there was no diet-related sex-biased survivorship (T+: GLM: LLR  $\chi^2=4.8397$ ,  $df=7$ ,  $p=0.680$ ; T-: GLM: LLR  $\chi^2=8.8596$ ,  $df=7$ ,  $p=0.2629$ ). Overall, we saw the flies' development speed depended on the type of experience in both experiments (Kruskal-Wallis test: T+: females:  $\chi^2=132.8$ ,  $df=7$ ,  $p<0.0001$ ; males:  $\chi^2=173.73$ ,  $df=7$ ,  $p<0.001$ ; Kruskal-Wallis test: T-: females:  $\chi^2=157.82$ ,  $df=7$ ,  $p<0.0001$ ; males:  $\chi^2=228.9$ ,  $df=7$ ,  $p<0.001$ ; Figure S2.3). In media that contained antimicrobials, flies developing on carbohydrate-rich media tended to eclose later than those on protein-rich media (Figure S2.3A,B), whereas, in the media that did not contain antimicrobials, the opposite pattern was seen (Figure S2.3C,D). Flies developing on different media (with antimicrobials) also eclosed at different mean masses (ANOVA: males:  $F_{7,340}=5.534$ ,  $p<0.001$ ; females:  $F_{7,378}=3.227$ ,  $p<0.001$ ). Pairwise comparisons of weights do not

suggest any specific directional pattern, except perhaps a tendency for flies in the extreme ratios eclosing at a lighter weight than others (Figure S2.4 and S2.6).

## **Discussion**

The Spotted-Wing fruit fly, *Drosophila suzukii*, is an invasive species responsible for a staggering amount of damage to agricultural efforts since its arrival in North America less than 10 years ago (Bolda *et al.* 2010). However, despite the significant economic toil that it has inflicted on host crops, there has been surprisingly little research conducted on host-preference behaviors or on the fitness consequences associated with the choice of oviposition host in this species. Here, using a series of assays based on a nutritional geometry framework, we explored host-association preferences in adult *D. suzukii*, and tested the suitability of different developmental environments to their offspring. We found strong preferences for media containing a relatively low protein: carbohydrate ratio for association and oviposition. We also found, in direct relation to the presence or absence of antimicrobials in the media, strong variation in larval survival and development across the P:C ratio spectrum. Whereas the greatest larval survival and development was found to be on media with high P:C ratios when antimicrobials were present, the opposite was found to be true when antimicrobials were absent. We explore the potential causes and consequences of these conflicting results from evolutionary, ecological and management perspectives.

In our first set of experiments, adult flies of both sexes were allowed to freely visit a variety of media that spanned a wide range of P:C ratios. We observed a significant non-random pattern in the physical location of both males and females, as well as in the number of eggs that were laid over the 25 hour observation period. Media with lower P:C ratios were consistently

visited more frequently (Figure S2.2), and were the site of greater oviposition, compared to media with higher P:C ratios (Figure 2.1, S2.5). Similarly, when individual females were placed into “no-choice” vials, those in the vials with low P:C media laid more eggs than those in the vials with high P:C media (Figure S2.6). Together, these results independently indicate a strong behavioral preference in adults for food medias that are rich in carbohydrates over those that are rich in proteins. The preference for high carbohydrates/low protein is not likely an artefact due to preferences that have evolved in the laboratory, as our media has a P:C ratio of ~1:3. A reason that flies exhibit this seeming preference may be due to the numerous metabolic benefits associated with this macronutrient (Maklakov *et al.* 2008; South *et al.* 2011). As is seen in other insects, the success of adult *D. sukukii* may depend on one’s ability to perform energy-demanding activities (Maklakov *et al.* 2008; South *et al.* 2011), such as rigorous courtship displays (Revadi *et al.* 2015) and daily foraging for mates, nutritional resources, and hosts. Carbohydrates, being a rapidly metabolized form of energy, may be preferred by *D. sukukii* for meeting their energy demands and/or optimizing performance. Our results are consistent with recent nutritional geometry studies by Rodrigues *et al.* (2015), Lihoreau *et al.* (2016), and Schwartz *et al.* (2016) that each found female *D. melanogaster* also laid a greater number of eggs on low P:C ratio media. However, while Lihoreau *et al.* (2016) found that *D. melanogaster* flies spent ~23% of their time on high protein foods (despite not ovipositing) we did not observe a similar association behavior in *D. sukukii*, where flies spent only ~6% of their time on the high P:C media. In many adult species, dietary protein is an important aspect for the stimulation of oogenesis and regulating vitellogenesis in females and for stimulating the production of sperm in males (Fenson *et al.* 2009; Fenson & Taylor 2012; Jensen *et al.* 2015; Lee *et al.* 2008; Lihoreau *et al.* 2016; Maklakov *et al.* 2008; Pirk *et al.* 2010; Reddiex *et al.* 2013), and thus it was

somewhat surprising to observe such a strong bias in activity and oviposition for low P:C medias in *D. suzukii*. Given the unique ecological niche *D. suzukii* occupies - parasitizing undamaged instead of damaged fruit - it is possible that adults may have evolved to require relatively less dietary protein compared to other species of *Drosophila*. Further studies on protein consumption in this species, may provide insight into the behaviors and dietary needs of *D. suzukii*, advancing our understanding of evolutionary processes, as well as furthering the management of this species.

Our oviposition results differ from the only previous study that examined oviposition-site choice in this species (Burrack *et al.* 2013). In that study, when flies were given a “choice” between raspberries (P:C ratio of ~1:4) and blueberries (P:C ratio of ~1:15), more eggs were laid on the former than on the latter. The reason for these differences may potentially be explained by differences in the experimental design of our two studies. Whereas we presented females with an artificial media for oviposition, Burrack *et al.*'s used intact blackberry, strawberry, blueberry and raspberry fruits. These various fruit differ in colour, aroma, size, texture, the force necessary to penetrate the skin (for example, blueberries require 3.4 times more force than raspberries; Burrack *et al.* 2013) and total macronutrient concentration (the P+C content of blueberry is ~2-fold higher raspberries at ~108 g/kg of fruit), any of which may have influenced their results. Our use of a standardized media potentially controlled for many of these confounding variables, we were able to reveal a previously unappreciated perspective to *D. suzukii* behavior.

Given the strong observed bias for increased oviposition on foods with low P:C ratios, we hypothesized that we would also see the greatest offspring success on this media, consistent with the Preference-Performance Hypothesis (PPH) or “Mother knows best” principle, in which female preference for oviposition is predicted to result in the highest offspring performance

(Gripenberg *et al.* 2010). Instead, in our third assay (in which we added antimicrobials to the media), we observed the opposite pattern of egg-to-adult survivorship and development rates as seen with the oviposition trend. The number of eggs that developed to adulthood on media with a low P:C ratio of 1:12 to 1:3 was markedly lower than those on media greater than 1:1 (Figure 2.2A, S2.7A,B). The apparent unsuitability of the high carbohydrate diets was also reflected in the slower development times of the flies on carbohydrate rich diets (Figure 2.2B). In fruit flies, more rapid development may allow adult flies to gain a competitive advantage over slower developing conspecifics when it comes to acquiring resources and/or finding mates (Markow and O'Grady 2005; Prasad *et al.* 2001) and, typically, survival is lower and developmental rates are slower in resource poor environments (e.g. Gebhardt and Stearns 1988; Edgar 2006). These results are suggestive that, from a developmental perspective, protein availability is of greater importance than carbohydrates for juvenile *D. suzukii* development. Interpreting the results of the body weight (Figure S2.4) is made more complicated by the fact that, in *Drosophila*, individual growth rates are mediated by the degree of intra-specific competition (reviewed by Ashburner *et al.* 2005). While flies on low P:C foods suffer greater mortality arising from the lower nutritional quality of their diet, those that do survive benefit from reduced competition, while for those flies developing in the high P:C media, the access to better nutrition may be offset by the increased pressure arising from increased competition for resources. To date, the only other study that has investigated offspring development in *D. suzukii* in the context of nutrition is by Jaramillo *et al.*'s (2015), who found no effect of P:C ratio in the larval diets to the egg-to-adult survivorship of larvae developing. However, that study was limited to only 2 different media types. Our use of nutritional geometry, a model developed by Simpson and Raubenheimer (1993), allowed us to dissect the effects that protein and carbohydrates have on life history traits in a more

comprehensive and standardized fashion, ultimately revealing a previously unknown aspect of this species.

The apparent conflict between the nutritional environment favored by *D. suzukii* adults and the types of resources that would most benefit their offspring would appear to be contrary to what one would have predicted according to the PPH (Gripenberg *et al.* 2010). The PPH has been tested in a number of insect species, and while several studies support this hypothesis, there are numerous cases where this relationship does not seem to apply (Gripenberg *et al.* 2010; Thompson 1988). For example, Lihoreau *et al.* (2016) observed that, in a similar nutritional geometry environment, female *D. melanogaster* “prefer” to lay more eggs on low P:C medias that are less supportive of larval development, than on high P:C medias, analogous to our observations with *D. suzukii*. Furthermore, females of the fruit fly species *D. koepferae* (Soto *et al.* 2012) the leaf galling insect *Neopelma baccharidis* (Faria and Fernandes 2001), and the Madrone butterfly *Eucheira socialis* (Underwood 1994), all show an oviposition “preference” for lower quality hosts. There are several potential explanations for the apparent conflict between optimal adult oviposition choice and offspring performance in *D. suzukii*. Firstly, it is possible that adult nutritional needs may trump the developmental needs of their offspring. Since reproduction and foraging are energy-demanding activities (Maklakov *et al.* 2008; South *et al.* 2011), adult dietary requirements may take a higher priority over those of juveniles and females may increase their fitness by being ‘selfish’ (e.g. Janz *et al.* 2005; Mayhew 2001; Scheirs & De Bruyn 2002). Evidence for this relationship among insects has been observed in the grass miner *Chromatomyia nigra* where females will lay eggs on the hosts they feed on, but not on hosts that are best for offspring performance (Scheirs 2000). Secondly, flexibility in adult behavior may be limited by the amount of genetic variation within the species for this trait. Without the necessary

additive genetic variation, *D. suzukii* may be unable to evolve adaptive oviposition behaviors and, thus, adults are ‘trapped’ on a carbohydrate-seeking trajectory. Considering that *D. suzukii* populations in North America may have descended from a small number of colonizing individuals, it is possible that flies in this region, as a result of a genetic bottleneck, have experienced a substantial reduction in allelic diversity (England *et al.* 2003). Alternatively, this apparent conflict may be due to factors associated with our experimental media. In Nature it is typical for microbial numbers in fruit to progressively multiply following oviposition by *D. suzukii* females (Hamby *et al.* 2012) and such progressions can be an important source of protein for fruit fly larvae (Begon 1986; Brito Da Cunha *et al.* 1951; Cooper 1959). Our experimental media (which followed the recipe of Lihoreau *et al.* 2016) which includes antimicrobial additives may have prevented the rapid growth of bacteria and yeasts, thereby removing a potential protein resource for developing larvae. Indeed, when raised on media without antimicrobial additives, in our follow-up experiment, we found larvae to exhibit greater survivorship on the high carbohydrate ratios, suggesting that *D. suzukii* ultimately benefit from the presence of microbes during development (Figure 2.3B, S2.7C,D). If such is the case, then the diet of juvenile *D. suzukii* may not have diverged substantially from other *Drosophila* species that parasitize rotting fruit (Begon 1986; Brito Da Cunha *et al.* 1951; Cooper 1959; Lihoreau *et al.* 2016). The fact that *D. suzukii* larvae fared best on our high-protein medias (with antimicrobials included) certainly seems to support this hypothesis. In regards to the PPH, a potential relationship between microbial-sourced proteins in rotting fruit and increased larval success would suggest a more harmonious association between the life-stages than our initial assay indicated. Furthermore, if suitable microbial growth is found to be greatest in fruits that are highest in carbohydrates, which females appear to find more attractive for egg-laying, the PPH may be highly relevant to



understanding the behaviour of this species. A strong oviposition preference for high carbohydrate foods observed in *D. suzukii*, coupled with the ability to capitalize on the microbial community as a source of protein may be an important reason for the success of this invasive species. As such, future studies on the relationship between juvenile success and microbial communities in hosts could yield important information on the factors that promote or inhibit offspring development and its relation to adult behavior and fitness.

*D. suzukii* is a serious economic pest and the progression of more effective control strategies is vital for avoiding large economic losses in the future. Our findings may be useful for farmers trying to decide on capture/control/removal strategies for various fruits that differ in P:C ratios. For example, from our experiments, it is clear that *D. suzukii* are strongly attracted to carbohydrate rich media for egg-laying, both in choice and in no-choice situations. Such behavior suggests that females will avoid laying eggs in habitats deemed unsuitable for oviposition and that the presence of carbohydrates and/or the absence of protein acts as an indicator of media suitability for oviposition. Fruits of lower P:C ratios, then, may act as both population “sinks” and “sources”, by drawing in more females and stimulating higher oviposition rates. In agroecosystems, it is generally considered that species live in a fragmented landscape where subpopulations are interconnected as a source-sink system (Tscharntke and Brandl 2004). Species richness and abundance is also dependent on local and landscape habitat characteristics (Rusch *et al.* 2013; Tscharntke and Brandl 2004; Weibull *et al.* 2003). Thus, the implementation of management strategies that incorporate population ecology based on the host landscape may be met with greater pest control efficiency and effectiveness (Ferreira *et al.* 2014; Gilioli *et al.* 2013). In terms of managing *D. suzukii*, farmers may incorporate methods that focus or implement more intensive management on fruit types in the landscape that act as population

“sources” and “sinks”. If we integrate our oviposition and survivorship data for our treatments in which antimicrobials were added (which inhibited microbial growth) we see that, all else being equal, fruit that consist of a P:C ratio of 1:3 to 1:6 are most likely to contribute the greatest number of surviving offspring (Figure 2.3A). In the situation where there is no inhibition of microbial growth, the integration of oviposition data and the survivorship in vials in which antimicrobials were omitted, fruit that is most likely to contribute the greatest number of surviving offspring consist of a P:C ratio of 1:12 and 1:6 (Figure 2.3B). Thus, farmers might do well to focus control efforts on crops in this P:C range. In order to implement this strategy to its greatest potential, insights from future studies looking at the antimicrobial properties of different fruit types and varieties could prove to be very valuable. In addition to incorporating a management strategy that is based on the existing population ecology demographics, growers may also enhance their results by altering the host landscape and manipulating fly movement. For instance, crop species may be used in the ‘push-pull’ strategy, whereby the distribution of a pest is manipulated for the purpose of management through the use of a combination of deterrent and attractive stimuli (Cook *et al.* 2007). In other words, the use of plants with fruits of a (more attractive) low P:C ratio may be used to deter or repel *D. suzukii* away (push) from the crop fruit, while, at the same time, lure flies (pull) into areas that contain traps or ‘trap crops’. Intercropping or the use of ‘cover crops’ is one way to incorporate this strategy, which involves that non-crop plant species, which modify pest behavior, are integrated with crop species. Growers may also incorporate the ‘push-pull’ strategy by organizing plants in the landscape so that they act as ‘barriers’. For example, plants that bear fruit of a low P:C ratio could be situated in areas surrounding the focal crop to prevent flies from entering into the crop area. Such a strategy would also allow management efforts to be focused on areas where pests are concentrated,

potentially reducing chemical pesticide use and, thus, the risk of insect resistance. In addition to incorporating a 'custom' management plan, the use of carbohydrates (e.g. corn syrup, juice etc.) in lures or baits may also improve the efficacy of attract-and-kill strategies, as adults were strongly attracted to the carbohydrate-rich medias in our experiment. Finally, from an ecological perspective, an association between larval success and microbial growth could have large implications on the population ecology of *D. suzukii*. For instance, different host fruits may vary in their susceptibility to the rate of microbial decay and/or the types of microbial life that it can support and, thus, contribute in various ways to population growth (e.g. if growth rate and different microbial species/taxa varies in its nutritional benefits). As such, a positive relationship between larval success and microbial colonization could be used to the farmers' benefit through the use of crops better able to resist microbial degradation (e.g. through genetic engineering) and/or the use of practices that reduce microbial colonization in fruit post-egg transfer (e.g. sterilization of plant and fruit exterior).

Nutritional geometry is an effective and well-established framework that is highly suitable for the investigation of targeted questions related to nutrition. By reducing the number of variables, the complexity of outcomes that accompany a complete diet is removed, allowing us to see how each nutritional variable affects life history traits, as well as to see how these variables interact. Our results highlight the importance that P:C ratios have on adult behavior and larvae performance in *D. suzukii*. Whereas, low P:C ratios were preferred by adults for association and oviposition, high P:C ratios provided the best nutritional environment for offspring. These results provide important insights into the foraging behaviors and nutritional needs of this pest species, which is important for improving current management.

## References

- Abraham., J., Zhang., A., Angeli., S., Abubeker., S., Michel., C., Feng., Y. and C. Rodriguez-Saona. 2015. Behavioral and antennal responses of *Drosophila suzukii* (Diptera: *Drosophilidae*) to volatiles from fruit extracts. *Environ Entomol.* 44(2): 356-367. doi:10.1093/ee/nvv013.
- Anderson., L. H., Kristensen., T. N., Loeschcke., V., Toft., S., and D. Mayntz. 2010. Protein and carbohydrate composition of larval food affects tolerance to thermal stress and desiccation in adult *Drosophila melanogaster*. *J Insect Physiol.* 56: 336-340. doi:10.1016/j.jinsphys.2009.11.006.
- Ashburner., M., Golic., K.G. and Hawley., R.S. 2005. *Drosophila: a laboratory handbook*, 2<sup>nd</sup> edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Atallah., J., Teixeira., L., Salazar., R., Zaragoza., G., and K. Artyom. 2014. The making of a pest: the evolution of a fruit-penetrating ovipositor in *Drosophila suzukii* and related species. *Proc R Soc B.* 281: 20132840. doi:10.1098/rspb.2013.2840.
- Begon, M. (1986) *Yeasts and Drosophila. The Genetics and Biology of Drosophila, Vol. 3b* (ed. by M.Ashburner, H.Carson and J. N.Thompson), pp. 345–383. Academic Press, London.
- Bellamy., E. B., Sisterson., M. S., and S. S. Walse. 2013. Quantifying Host Potentials: Indexing Postharvest Fresh Fruits for Spotted Wing *Drosophila*, *Drosophila suzukii*. *PLoS ONE* 8(4): e61227. doi:10.1371/journal.pone.0061227.
- Bolda., M. P., Goodhue., R. E., and F. G. Zalom. 2010. Spotted wing drosophila: potential economic impact of a newly established pest. *Giannini Foundation of Agricultural Economics*, University of California.
- Burrack., H. J., Fernandez., G. E., Spivey., T., and D. A. Kraus. 2013. Variation in selection and utilization of host crops in the field and laboratory by *Drosophila suzukii* Matsumara (Diptera: *Drosophilidae*), an invasive frugivore. *Pest Manag Sci.* 69: 1173-1180. doi 10.1002/ps.3489.
- John Fox and Sanford Weisberg (2011). *An {R} Companion to Applied Regression*, Second Edition. Thousand Oaks CA: Sage.  
URL:<http://socserv.socsci.mcmaster.ca/jfox/Books/Companion>
- Carrel., J. E., and E. M. Tanner. 2002. Sex-specific food preferences in the Madagascar hissing cockroach *Gromphadorhina portentosa* (Dictyoptera: *Blaberidae*). *J Insect Behav.* 15(5): 707-714. doi:10.1023/A:1020704108399.

Cook., M. C., Khan., Z. R., and J. A. Pickett. 2007. The use of push-pull strategies in integrated pest management. *Annu Rev Entomol.* 52: 375-400. doi:10.1146/annurev.ento.52.110405.091407.

Cooper., D. M. 1959. Food preferences of larval and adult *Drosophila*. *Evolution*, 14, 41–55.

Da Cunha., B., Dobzhansky., T., and A. Sololoff. 1951. On food preferences of sympatric species of *Drosophila*. *Evolution.* 5: 97-101.

Edgar., B. A. 2006. *How flies get their size: genetics meets physiology*. *Nat Rev Genet* 7: 907-916

England., P. R., Osler., G. H. R., Woodworth., L. M., Montgomery., M. E., Briscoe., D. A., and R. Frankham. 2003. Effects of intense versus diffuse population bottlenecks on microsatellite genetic diversity and evolutionary potential. *Conserv Genet.* 4: 595-604.

Faria., M. L., and G. W. Fernandes. 2001. Vigour of a dioecious shrub and attack by a galling herbivore. *Ecol Entomol.* 26: 37-45.

Ferreira., C., P., Esteva., L., Godoy., W. A. C., and F. L. Consoli. 2014. Landscape diversity influences dispersal and establishment of pest with complex nutritional ecology. *Bull Math Biol.* 76: 1747-1761. doi:10.1007/s11538-014-9975-1.

Gebhardt., M. D., and C. Stearns 1988. Reaction norms for developmental time and weight at eclosion in *Drosophila mercatorum*. *J Evol Biol.*

Gilioli., G., Bodini., A., and J., Baumgartner. (2013) Metapopulation modelling and area-wide pest management strategies evaluation. An application to the Pine processionary moth. *Ecol Modell.* 260:1–10.

Gripenberg., S., Mayhew., P. J., Parnell., M., and T. Roslin. 2010. A meta-analysis of preference-performance relationships in phytophagous insects. *Ecol Lett.* 13: 383-393. doi:10.1111/j.1461-0248.2009.01433.x.

Hamby., K. A., Hernandez., A., Boundy-Mills., K., and F. G. Zalom. 2012. Associations of yeasts with Spotted-wing *Drosophila* (*Drosophila suzukii*; Diptera: *Drosophilidae*) in cherries and raspberries. *Appl and Environ Microbiol.* 78 (14): 4869-4873. doi:10.1128/AEM.00841-12.

Hanks., L. M., Paine., T. D., and J. G. Miller. 1993. Host species preference and larval performance in the wood-boring beetle *Phoracantha semipunctata* F. *Oecol.* 95: 22-29.

Jaramillo., S. L., Mehlferber., E., and P. J. Moore. 2015. Life-history trade-offs under different larval diets in *Drosophila suzukii* (Diptera: *Drosophilidae*). *Physiol Entomol.* 40: 2-9. doi:10.1111/phen.12082.

Janz., N., Bergstrom., A., and A. Sjogren. 2005. The role of nectar sources for oviposition decisions of the common blue butterfly *Polyommatus icarus*. *Oikos.* 109, 535–538.

Jensen., K., McClure., C., Priest., N. K., and J. Hunt. 2015. Sex-specific effects of protein and carbohydrate intake on reproduction but not lifespan in *Drosophila melanogaster*. *Aging Cell.* 14: 605-615. doi:10.1111/accel.12333.

Konig., M. A. E., Wiklund., C., and J. Ehrlen. 2016. Butterfly oviposition preference is not related to larval performance on a polyploid herb. *Ecol Evol.* 6(9): 2781-2789. doi:10.1002/ece3.2067.

Lee., K. P., Simpson., S. J., Clissold., F. J., Brooks., R., Ballard., J. W. O., Taylor., P. W., Soran., N., and D. Raubenheimer. 2008. Lifespan and reproduction in *Drosophila*: New insights from nutritional geometry. *PNAS.* 105(7): 2498-2503.

Lee., J. C., Bruck., D. J., Curry., H., Edwards., D., Haviland., D. R., Steenwyk., R. A., and B. M. Yorgey. 2011. The susceptibility of small fruits and cherries to the spotted-wing drosophila, *Drosophila suzukii*. *Pest Manag Sci.* 67: 1358-1367.

Lefranc., A., and J. Bundgaard. 2000. Controlled variation of body size by larval crowding in *Drosophila melanogaster*. *Dros Inf Serv.* 83: 171-174.

Lihoreau., M., Poissonnier., L., Isabel., G., and A. Dussutour. 2016. *Drosophila* females trade off good nutrition with high quality oviposition sites when choosing foods. *J Exp Biol.* 219(16): 2514-24. doi:10.1242/jeb.142257.

Maklakov., A. A., Simpson., S. J., Zajitschek., F., Hall., M. D., Dessmann., J., Clissold., F., Raubenheimer., D., Bonduriansky., R., and R. C. Brooks. 2008. Sex-specific fitness effects of nutrient intake on reproduction and lifespan. *Current Biology.* 18: 1062-1066. doi:10.1016/j.cub.2008.06.059.

Rose., M. R. 1984. Laboratory evolution of postponed senescence in *Drosophila melanogaster*. *Evolution.* 1004-1010.

Markow., T.A. and O'Grady., P.M. 2005. *Drosophila: A Guide to Species Identification and Use.* 250pp. Academic Press, London.

Markow., T. A. 2015. The natural history of model organisms: the secret lives of *Drosophila* flies. eLife. doi:10.7554/eLife.06793.

May., C. M., Doroszuk., A., and B. J. Zwaan. 2015. The effect of developmental nutrition on life span and fecundity depends on the adult reproductive environment in *Drosophila melanogaster*. Ecol Evol. 5(6):1156-1168. doi: 10.1002/ece3.1389.

Mayhew., P. J. 1997. Adaptive patterns of host plant selection by phytophagous insects. Oikos. 79, 417–428.

Morimoto., J., and S. Wigby. 2016. Differential effects of male nutrient balance on pre- and post-copulatory traits, and consequences for female reproduction in *Drosophila melanogaster*. Sci Rep. 6: 27673. doi:10.1038/srep27673.

Giraudoux, P. 2016. pgirmess: Data Analysis in Ecology. R package version 1.6.5. <https://CRAN.R-project.org/package=pgirmess>.

Partridge., L., Hoffman., A., and J. S. Jones. 1987. Male size and mating success in *Drosophila melanogaster* and *D. pseudoobscura* under field conditions. Anim Behav. 35: 468-476.

Partridge., L., Ewing., A., and A. Chandler. 1987. Male size and mating success in *Drosophila melanogaster*: the roles of male and female behavior. Anim Behav. 35: 555-562.

Pirk., C. W. W., Boodhoo., C., Human., H., and S. W. Nicolson. 2010. The importance of protein type and protein to carbohydrate ratio for survival and ovarian activation of caged honeybees (*Apis mellifera scutellata*). Apidologie. 41: 62-72. doi:10.1051/apido/2009055.

Prasad., N. G., Shakarad., M., Anitha., D., Rajamani., M. and A. Joshi. 2001. Correlated responses to selection for faster development and early reproduction in *Drosophila*: the evolution of larval traits. Evolution 55.7: 1363-1372

Raubenheimer., D., and S. J. Simpson. 2003. Nutrient balancing in grasshoppers: behavioural and physiological correlates of dietary breadth. J Exp Biol. 206: 1669-1681. doi:10.1242/jeb.00336.

Rausher., M. D. 1979. Larval habitat suitability and oviposition preference in three related butterflies. Ecol. 60(3): 503-511.

R Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reddiex., A. J., Gosden., T. P., Bonduriansky., R. and S. F. Chenoweth. 2013. Sex-specific fitness consequences of nutrient intake and the evolvability of diet preferences. *Amer Nat.* 182 (1): 91-102. doi:10.1086/670649.

Revadi., S., Lebreton., S., Witzgall., P., Anfora., G., Dekker., T., and P. G. Becher. 2015. Sexual behavior of *Drosophila suzukii*. *Insects.* 6: 183-196. doi:10.3390/insects6010183

Rodrigues., M. A., Martins., N. E., Balance., L. F., Broom., L. N., Dias., A. J. S., Fernandes., A. S. D., Rodrigues., F., Sucena., E., and C. K. Mirth. 2016. *Drosophila melanogaster* larvae make nutritional choices that minimize developmental time. *J Insect Physiol.* 81: 69-80.

Rusch A, Valantin-Morison M, Sarthou JP, Rogeer-Estrade J. 2013. Effect of crop management and landscape context on insect populations and crop damage. *Agric Ecosyst Environ.* 166:118–125.

Scheirs, J., De Bruyn, L. and Verhagen, R. 2000. Optimization of adult performance determines host choice in a grass miner. *Proc R Soc Lond B.* 267: 2065–2069.

Scheirs, J. & De Bruyn, L. 2002. Integrating optimal foraging and optimal oviposition theory in plant–insect research. *Oikos.* 96, 187–191.

Schwartz., N. U., Zhong., L., Bellemer., A. and W. D. Tracey. 2012. Egg laying decisions in *Drosophila* are consistent with foraging costs of larval progeny. *PLoS ONE.* 7(5). doi:10.1371/journal.pone.0037910

Simpson., S. J., and D. Raubenheimer. 1993. A multi-level analysis of feeding behavior: the geometry of nutritional decisions. *Phil Trans R Soc Lond B.* 342: 381-402.

Soto., E. M., Goenaga., J., Hurtado., J. P., and E. Hasson. 2012. Oviposition and performance in natural hosts in cactophilic *Drosophila*. *Evol Ecol.* 26: 975-990. doi:10.1007/s10682-011-9531-5.

Tantawy., A. O., and M. O. Vetukhiv. 1960. Effects of size on fecundity, longevity and viability in populations of *Drosophila pseudoobscura*. *Amer Nat.* 94(879): 395-403.

Thomas H. T. 1993. Ecology of body size in *Drosophila buzzatii*: untangling the effects of temperature and nutrition. *Ecol Entomol.* 18: 84-90. doi: 10.1111/j.1365-2311.1993.tb01084.

Tscharntke, T., and R. Brandl. 2004. Plant-insect interactions in fragmented landscapes. *Annu Rev Entomol.* 49, 405–430.



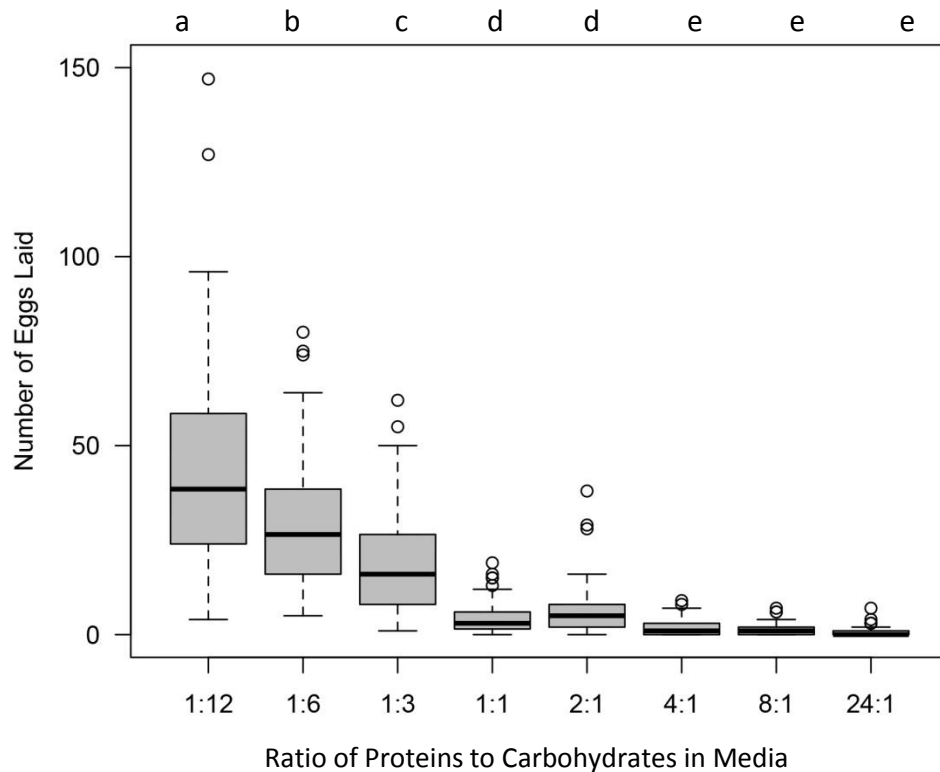
Underwood., D. L. A. 1994. Intraspecific variability in host plant quality and ovipositional preferences in *Eucheira socialis* (Lepidoptera: *Pieridae*). *Ecol Entomol.* 19: 245-256.

(USDA) U.S. Department of Agricultural National Agricultural Statistics Service. 2009. noncitrus fruits and nuts 2008 summary. publication fruit and nuts 1–3 (09). U.S. Department of Food Agriculture of National Agricultural Statistics Service, Washington, D.C.

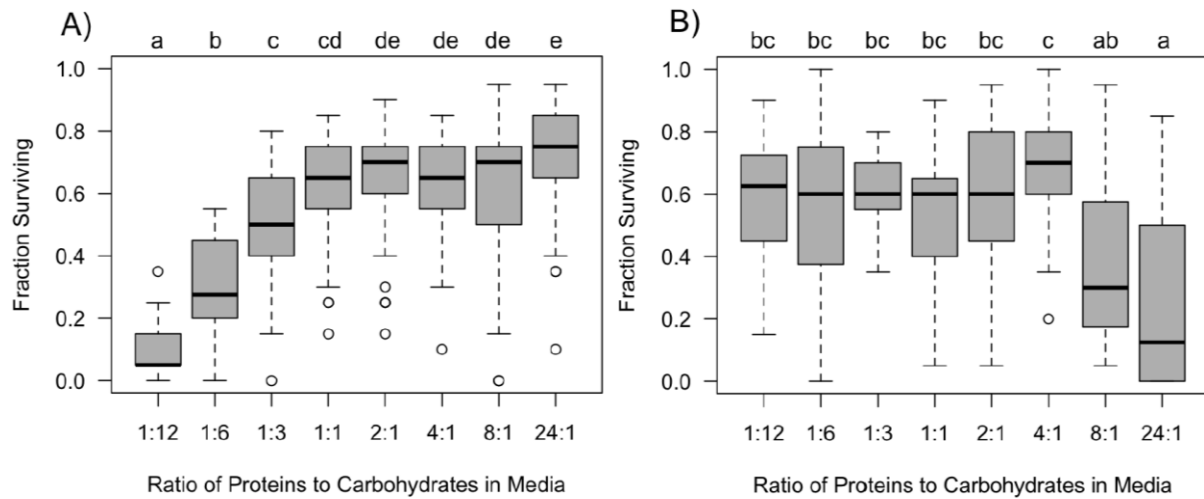
Walsh., D. B., Bolda., M. P., Goodhue., R. E., Dreves., A. J., Lee., J., Bruck., D. J., Walton., V. M., O’Neal., S. O., and F. G. Zalom. 2011. *Drosophila suzukii* (Diptera: *Drosophilidae*): Invasive pest of ripening soft fruit expanding its geographic range and damage potential. *J Integ Pest Mngmt.* 2(1): doi:10.1603/IPM10010

Weibull, A.C., Ostman, O., Granqvist, A., 2003. Species richness in agroecosystems: the effect of landscape, habitat and farm management. *Biodivers Conserv.* 12, 1335–1355.

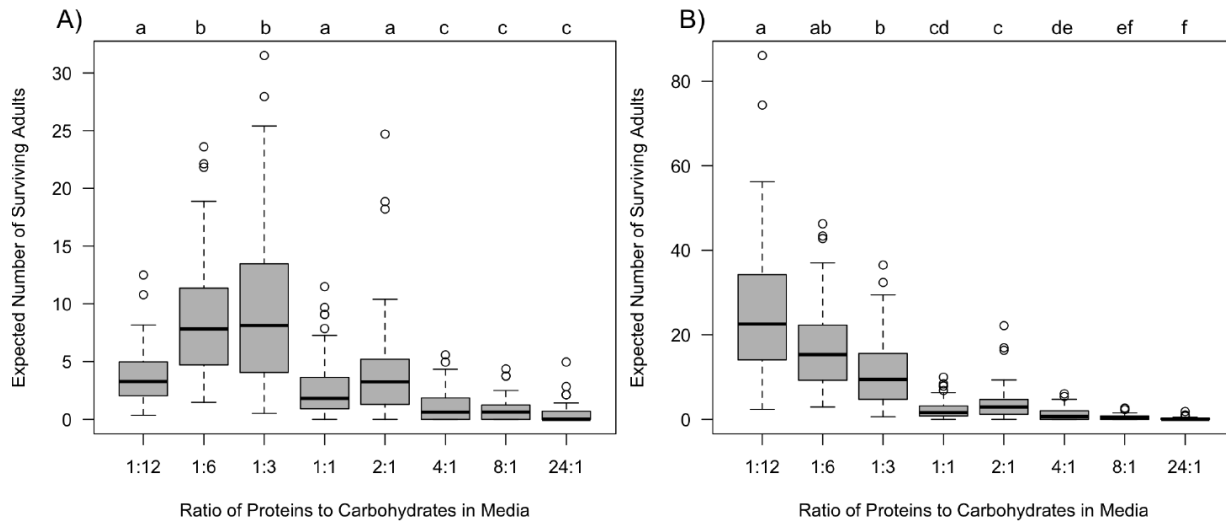
## Figures



**Figure 2.1.** Oviposition preference among medias differing in protein: carbohydrate (P:C) ratio in choice environments. Boxplots of numbers of eggs female *D. sukukii* laid on each of the eight different P:C medias in each choice chamber (80 chambers in total) over a 25-hour period. The box encloses values between the first and third quartiles of the data (the inter-quartile range (IQR)), whereas the horizontal bar within the box indicates the median. Whiskers extend from the box to largest/smallest values that are within  $1.5 \times$  the IQR of the box. Values outside that range are outliers and are indicated by circles. Boxplots that are not sharing a letter have significantly different means.



**Figure 2.2.** Larval survivorship on medias differing in protein: carbohydrate (P:C) ratio. Boxplots of proportion of flies that eclosed as adults within 22 days following the transfer of a standardized number of eggs (20) into vials containing artificial media A) with antimicrobials and B) without antimicrobials. The box encloses values between the first and third quartiles of the data (the inter-quartile range (IQR)), whereas the horizontal bar within the box indicates the median. Whiskers extend from the box to largest/smallest values that are within  $1.5 \times$  the IQR of the box. Values outside that range are outliers and are indicated by circles. Boxplots that are not sharing a letter have significantly different means.



**Figure 2.3.** Predicted number of surviving adults on medias differing in protein: carbohydrate (P:C) ratio. Boxplot of number of adults predicted to eclose on substrates of differing P:C ratios based on oviposition rates in a choice environment and survivorship values derived from the assays in media, A) inhibited microbial growth and B) supported microbial growth. In both analyses there was significant differences in the number of expected offspring eclosing from each media type (Kruskal-Wallis tests: A:  $\chi^2=356.01$ ,  $df=7$ ,  $p<0.0001$ ; B:  $\chi^2=476.45$ ,  $df=7$ ,  $p<0.001$ ). The box encloses values between the first and third quartiles of the data (the interquartile range (IQR)), whereas the horizontal bar within the box indicates the median. Whiskers extend from the box to largest/smallest values that are within  $1.5 \times$  the IQR of the box. Values outside that range are outliers and are indicated by circles. Boxplots that are not sharing a letter have significantly different medians.

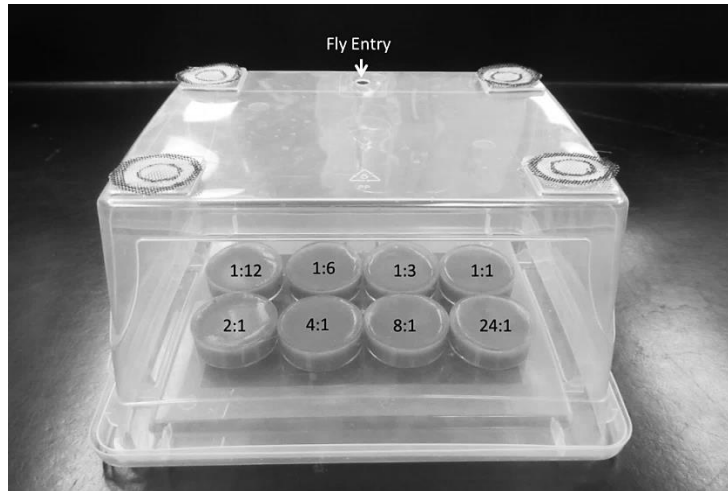
### Supplementary Tables & Figures

**Supplemental Table 2.1.** The protein to carbohydrate ratios and concentrations of fruit species attacked by *D. suzukii* in North America. Nutritional information accessed from the USDA Nutrient Database (<https://ndb.nal.usda.gov/ndb/>).

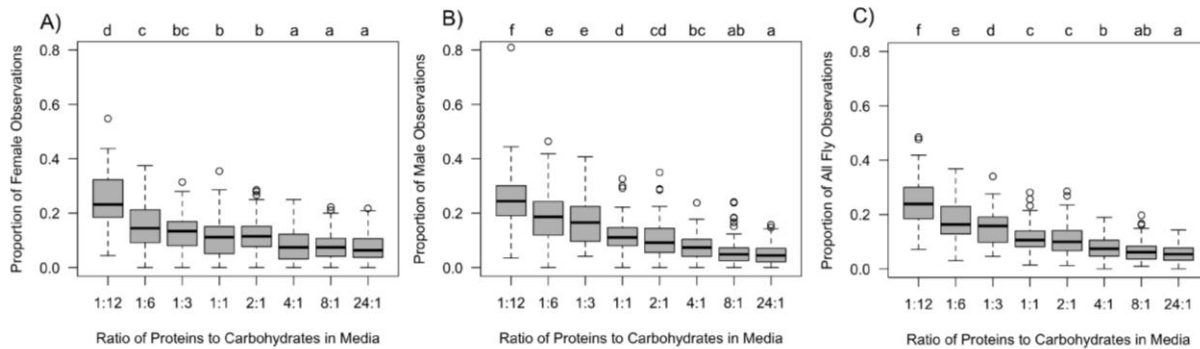
Species	Protein: Carbohydrate	Protein + Carbohydrate (g/kg)
Blueberry ( <i>Vaccinium spp.</i> )	1:15	108
Plum ( <i>Prunus spp.</i> )	1:14	107
Elderberry ( <i>Sambucus spp.</i> )	1:10	77
Peach ( <i>Prunus persica</i> )	1:9	89
Sweet Cherry ( <i>Prunus spp.</i> )	1:8	90
Nectarine ( <i>Prunus persica</i> )	1:7	91
Sour Cherry ( <i>Prunus spp.</i> )	1:7	96
Strawberry ( <i>Fragaria spp.</i> )	1:7	56
Apricot ( <i>Prunus spp.</i> )	1:6	104
Mulberry ( <i>Morus spp.</i> )	1:6	94
Currant ( <i>Ribes spp.</i> )	1:5	84
Gooseberry ( <i>Ribes spp.</i> )	1:5	52
Raspberry ( <i>Rubus spp.</i> )	1:5	49
Chokecherry ( <i>Prunus virginiana</i> )	1:5	169
Blackberry ( <i>Rubus spp.</i> )	1:4	63
Grape ( <i>Vitis spp.</i> )	1:3	220

**Supplemental Table 2.2.** Exact quantities of protein (P) and carbohydrates (C) in each 1 L ratio recipe.

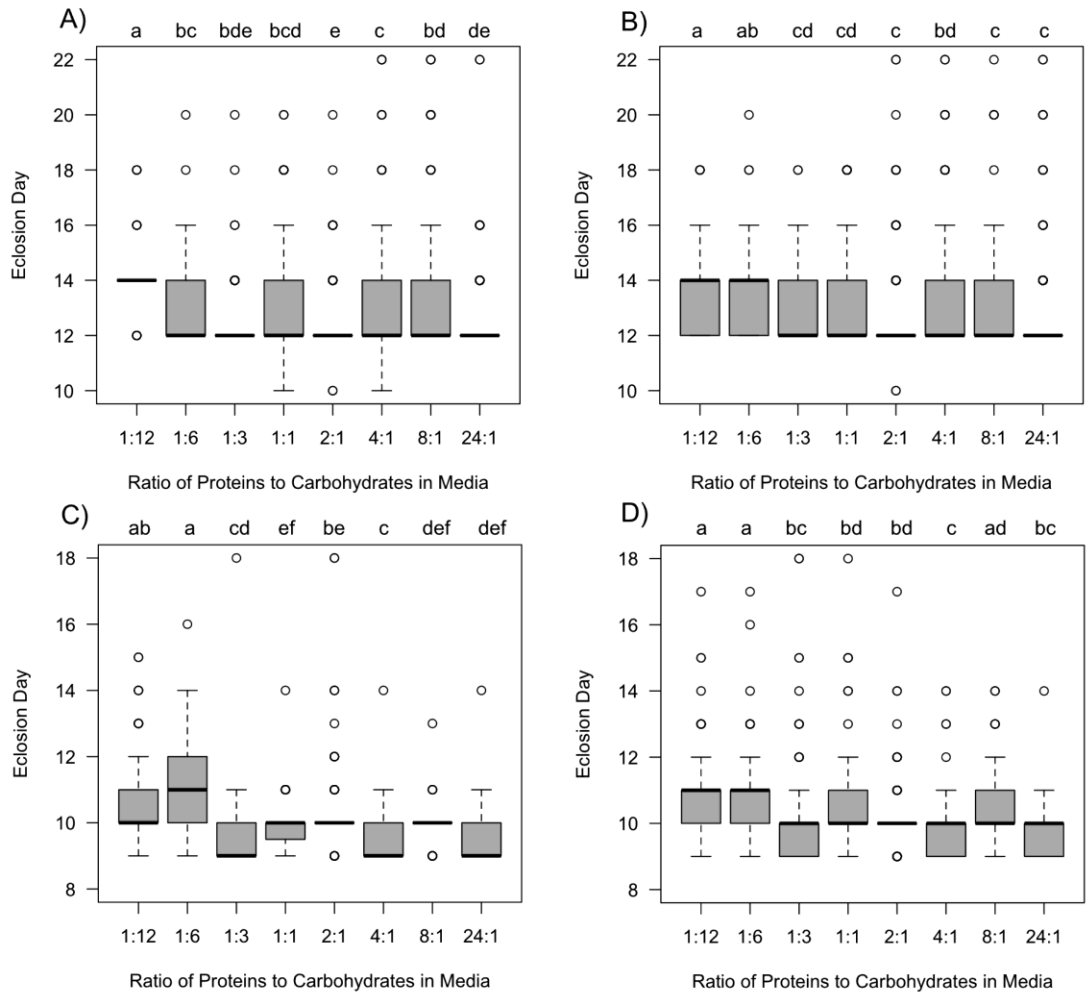
Ratio P:C	1:12	1:6	1:3	1:1	2:1	4:1	8:1	24:1
Protein (g/L)	5	10	18	35	47	56	62	67
Carbohydrates (g/L)	65	60	53	35	23	14	8	3



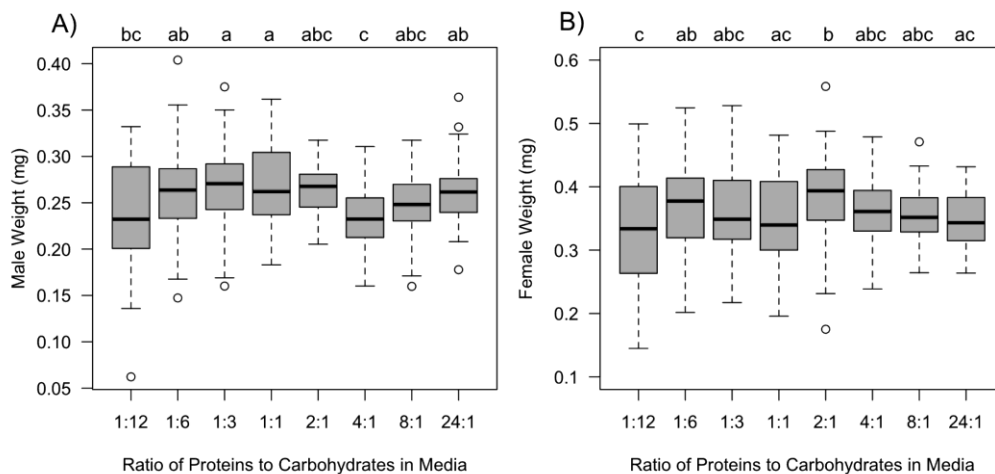
**Supplementary Figure 2.1.** Picture of a “choice” chamber containing dishes of the eight different protein-to-carbohydrate (P:C) ratio medias.



**Supplementary Figure 2.2.** Boxplots of distribution of flies among medias differing in protein to carbohydrate ratio (P:C). Proportions of time *Drosophila suzukii* females (A), males (B) and both sexes together (C) spent on each of the eight different P:C medias in the choice chambers at any one time over a 26-hour observation period. The box encloses values between the first and third quartiles of the data (the inter-quartile range (IQR)), whereas the horizontal bar within the box indicates the median. Whiskers extend from the box to largest/smallest values that are within  $1.5 \times$  the IQR of the box. Values outside that range are outliers and are indicated by circles. Boxplots that are not sharing a letter have significantly different means.

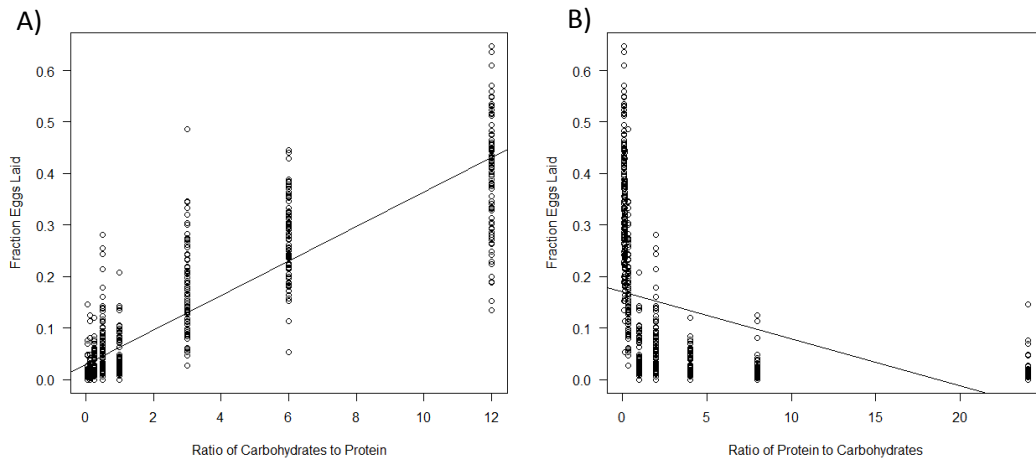


**Supplementary Figure 2.3.** Boxplots of dates of eclosion from pupae of adult *Drosophila suzukii* on medias differing in their protein: carbohydrate (P:C) ratio. Left panels (A & C) indicate observations made for males, while right panels (B & D) indicate observations made for females. Top panels (A & B) represent data collected from the assay in which antimicrobials were added to media while bottom panels (C & D) represent data collected from the assay in which antimicrobials were omitted from the media. The box encloses values between the first and third quartiles of the data (the inter-quartile range (IQR)), whereas the horizontal bar within the box indicates the median. Whiskers extend from the box to largest/smallest values that are within  $1.5 \times$  the IQR of the box. Values outside that range are outliers and are indicated by circles. Boxplots that are not sharing a letter have significantly different medians.

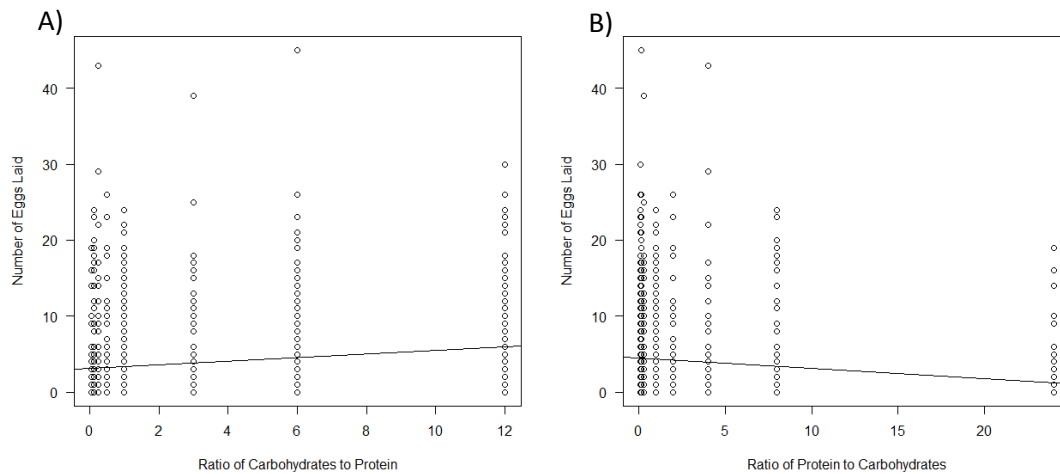


**Supplementary Figure 2.4.** Boxplot of weights of adult male (A) and female (B) *Drosophila suzukii* raised on media differing in protein: carbohydrate (P:C) ratio (with antimicrobials). The box encloses values between the first and third quartiles of the data (the inter-quartile range (IQR)), whereas the horizontal bar within the box indicates the median. Whiskers extend from the box to largest/smallest values that are within  $1.5 \times$  the IQR of the box. Values outside that range are outliers and are indicated by circles. Boxplots that are not sharing a letter have significantly different means.

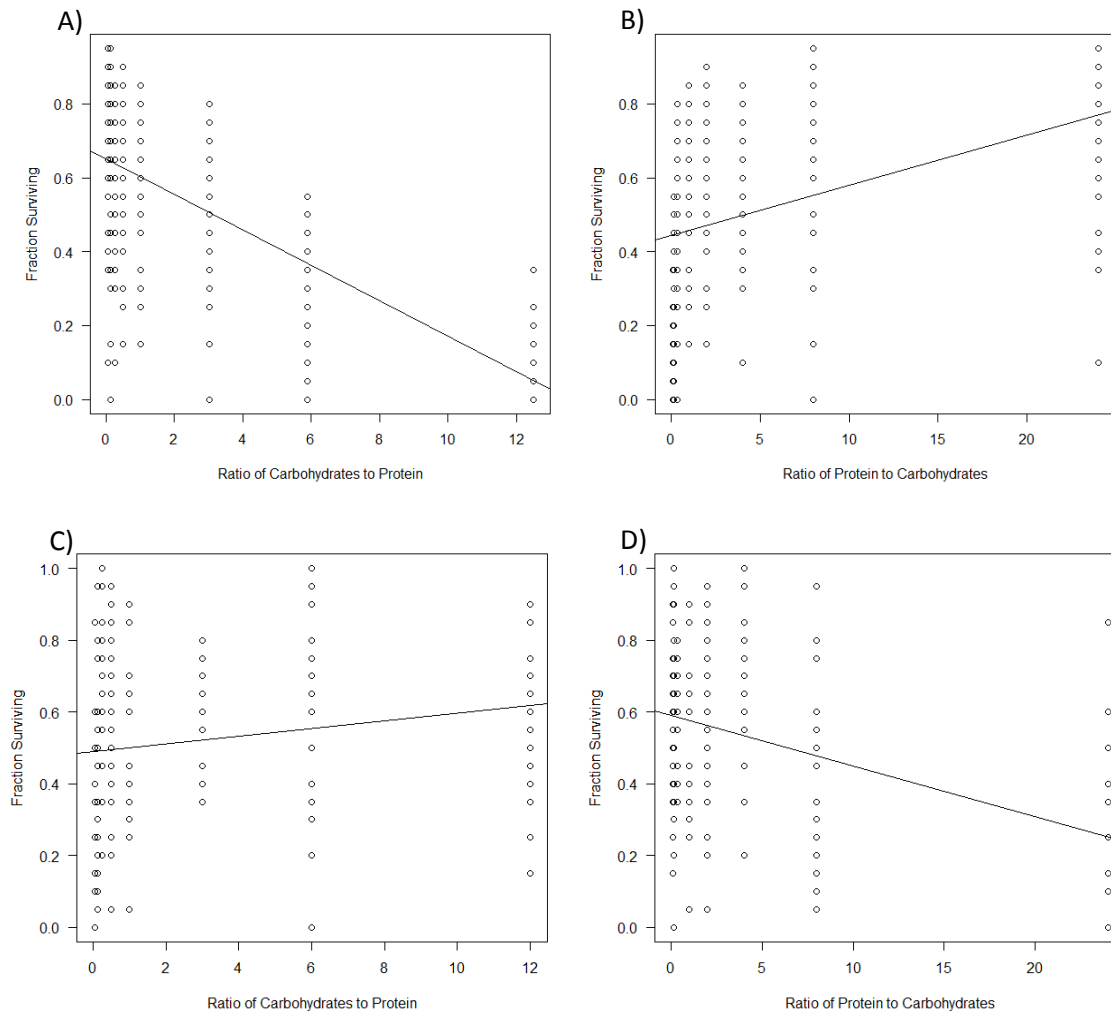




**Supplementary Figure 2.5.** Scatter plots illustrating the regression lines between the total number of eggs laid in the ‘choice’ scenario in relationship to A) the carbohydrate to protein ratio in the media (slope of 0.033 and y-intercept of 0.029) and B) the protein to carbohydrate ratio in the media (slope of -0.009 and y-intercept of 0.169).



**Supplementary Figure 2.6.** Scatter plots illustrating the regression lines between the total number of eggs laid in the ‘no choice’ scenario in relationship to A) the carbohydrate to protein ratio in the media (slope of 0.238 and y-intercept of 3.095) and B) the protein to carbohydrate ratio in the media (slope of -0.013 and y-intercept of 4.45).



**Supplementary Figure 2.7.** Scatter plots illustrating the regression lines between the proportion of offspring surviving in relationship to A) the carbohydrate to protein ratio in the media with *antimicrobials added* (slope of -0.048 and y-intercept of 0.652), B) the protein to carbohydrate ratio in the media with *antimicrobials added* (slope of 0.44 and y-intercept of 0.014), C) the carbohydrate to protein ratio in the media with *antimicrobials omitted* (slope of 0.490 and y-intercept of 0.011), and D) the protein to carbohydrate ratio in the media with *antimicrobials omitted* (slope of -0.014 and y-intercept of 0.591).

**CHAPTER 3**

**NUTRITIONAL DEVELOPMENTAL HISTORY AND ITS CONSEQUENCES FOR  
REPRODUCTIVE SUCCESS IN DROSOPHILA SUZUKII**

Yvonne Young & Tristan A.F. Long

## **Preamble**

The following chapter was written as a manuscript in the style of *Animal Behavior*, where it will be submitted for consideration shortly.

## **Abstract**

The characteristics of an individual's juvenile developmental environment can have important consequences for their adult reproductive success as it may shape the development and expression of phenotypes that are relevant to the operation of sexual selection. In complex landscapes, species may exhibit phenotypic plasticity that enables individuals to maximize their reproductive potential. *Drosophila suzukii* is an economically important invasive pest species that lays its eggs in many types of soft-fruits and, consequently experiences large intra-population spatial and temporal variation in its nutritional developmental environment. Here, we examine whether the developmental environment influences *D. suzukii* mate choice and offspring performance. Using flies raised on either a low or high "quality" diet we examined mating preferences, fecundity and offspring survivorship in no-choice, female choice and male choice contexts. Overall, we found, depending on the environmental context, evidence for adaptive and non-adaptive mate choice behaviors that were associated with the phenotypes of flies that had developed on different media. These results reveal the complex nature of the relationship between the developmental environment and individual reproductive success in *D. suzukii*, which has important potential implications for future management plans involving this pest species.

## Introduction

Sexual selection theory posits that mate preference(s) should evolve if individuals of one sex vary in quality, or reproductive potential, thereby making ‘choosy’ behavior beneficial for improving fitness relative to a random-mating strategy (reviewed by Kokko *et al.* 2003; Promislow 1998). The intensity of ‘choosiness’ during mate selection is often associated with the life history of the species in that the more cost there is associated with making a ‘bad’ mate choice, the greater the advantage of being choosy. Central to every sexual selection model is the idea that there is a fitness benefit associated with being ‘choosy’ (reviewed by Kokko *et al.* 2003; Bateson 1984) and many studies have attempted to measure these benefits (Head *et al.* 2005; Petrie 1994; Reynolds and Gross 1992; Ryan and Altmann 2001; Simmons 1987). Benefits can be *direct* and involve the production of more and/or better quality offspring. For instance, common measurements of fitness among insects include the number of eggs produced following copulation and the survivorship of the offspring to adulthood. Benefits can also be *indirect* in that the fitness of the offspring is increased (Kokko *et al.* 2003). Such measurements typically include the mating attractiveness of the offspring (e.g. ‘sexy son’ hypothesis; Weatherhead and Robertson 1981).

One factor with the potential to amplify these costs, and thus increase sexual selection pressures on a species, is that of the local environment (Awmack and Leather 2002; Botero and Rubenstein 2012; Chain and Lyon 2008; Cockburn *et al.* 2008; Gardner *et al.* 2009; Miller and Svensson 2013). By shaping individual traits that are highly relevant to the fitness of the organism, the developmental environment can influence the phenotypes of individuals, which has substantial consequences for individual fitness variation. In species experiencing large phenotypic and/or behavioral variation on a regular basis there may be strong selection to choose

partners that will provide the greatest fitness benefits (Botero and Rubenstein 2012; Cotton *et al.* 2006; Miller and Svensson 2013). This relationship, where greater phenotypic variation results in greater ‘choosiness’ by individuals for partners, has been observed in a number of species including the black field cricket (*Teleogryllus commodus*; Hunt *et al.* 2005), the stalk-eyed fly (*Telopsis dalmanni*; Hingle *et al.* 2001), the deep snouted pipefish (*Syngnathus typhle*; Mazzi 2004) and the fruit fly (*Drosophila melanogaster*; Sharon *et al.* 2010; Najarro *et al.* 2015; Dodd 1989; Abed-Vieillard *et al.* 2016). In all cases we see strong selection to choose mates able to provide the best fitness advantage.

*Drosophila suzukii*, the spotted-wing fruit fly, an invasive and economically important agricultural pest to North America, Europe and South America (reviewed by Asplen *et al.* 2015), is a species that inhabits (with apparent ease) many different environments. These environments not only vary spatially, encompassing cities, towns, agricultural areas and natural environments, but also vary temporally in the resources that they provide. As a generalist parasite of thin-skinned fruits (Asplen *et al.* 2015), which includes commercial crops such as blueberries, blackberries, strawberries, raspberries and occasionally grapes (Bellamy *et al.* 2013; Lee *et al.* 2011) and numerous wild and ornamental fruits (Heimpel *et al.* 2010; Walsh *et al.* 2011), *D. suzukii* potentially experiences large heterogeneity in the types of fruits available to them for ovipositing. In insects, it is well known that the nutritional environment during development can play a significant role in determining adult phenotype and/or behavior (Awmack and Leather 2002; Etges *et al.* 2006; Fedina *et al.* 2012; Schultzhaus *et al.* 2017) which ultimately has consequences for an individual’s potential reproductive success (Awmack and Leather 2002; Gardner *et al.* 2009; Padilla and Adolph 1996). In *D. suzukii*, since larvae develop in different fruit species and fruit species vary in their nutritional composition and quality, this means that

populations potentially consist of a wide range of different adult phenotypes. Indeed, host-induced variation in the adult size of *D. suzukii* females has been observed in individuals raised on various fruit-based medias. In the study by Bellamy *et al.* (2003) it was found that females raised on a cherry substrate and a raspberry substrate were significantly larger than females raised on a blackberry, blueberry, grape or peach substrate. We also observed differences adult size in our nutritional geometry experiment (Chapter 2) where larvae that developed on medias consisting of the highest and lowest protein-to-carbohydrate ratio (1:12 and 24:1) were smaller than those in the mid-ranges. Larger size in females in insects is often associated with increased fecundity and fertility (Honek 1993), suggesting that *D. suzukii* females may experience variation in fecundity as a direct result of their specific diet during development. As such, it is worth considering the importance of sexual selection in situations where the quality of potential mates differ, and it is possible that *D. suzukii*'s success as an invasive pest may be associated with its ability to distinguish and choose mates that are of higher quality, thus maximizing individual fitness. Observations made by Diepenbrock *et al.* (2016) suggest that *D. suzukii* alters its behavior to improve individual fitness based on prior experience with the host environment. In this study, females that developed on fruits of lower nutritional quality demonstrated behavioral plasticity in egg-laying by preferring to oviposit on higher quality hosts. Juvenile experience with hosts has also been found to modulate behaviors in a number of other insect species (reviewed by Anderson and Anton 2014). In terms of sexual selection, *D. suzukii* exhibit a number of traits commonly associated with species that have strong mate preferences. For example, male *D. suzukii* use highly sophisticated courtship displays, involving elaborate movements with their sex-specific, single-spotted wings (Revadi *et al.* 2015) and substrate-borne vibrations (Mazzoni *et al.* 2013), which, in other *Drosophila* species, are used to communicate



information about a male's reproductive quality and entice females into mating (reviewed by Ewing 1983). *Drosophila suzukii* also release a “perfume of pheromones” (cuticular hydrocarbons), which are thought to advertise sexual maturity and other aspects of mate “quality” in other *Drosophila* and function as a chemical cue during mate choice (reviewed by Ferveur 2005). By investigating sexual selection in *D. suzukii* we may gain a better understanding of how environmental heterogeneity influences sexual selection in this species and also gain insight into the factors that enable this pest to be so successful in foreign and complex environments- information which may be useful for improving control methods of *D. suzukii*. For instance, knowledge on the male phenotypic characters that are found attractive to females may be used to improve the attractiveness of reared male flies for release as part of the sterile insect technique, a management strategy that aims to reduce population growth through the facilitation of matings between sterile and wild individuals (Klassen and Curtis 2005). As well, knowledge on individual fitness of flies of particular phenotypes may be used to plant host crops in configurations that produce phenotypic combinations that are of lower fitness (e.g., reduced egg production following mating with a particular phenotype).

Mating behavior in organisms is based on the presence of relevant cues (e.g., pheromones, colour, vocalizations) that are recognizable and can be used to determine whether an individual is worth pursuing/accepting as a potential mate (Kokko *et al.* 2003). Adding complexity to this mate assessment system is the modification of these sexually relevant cues by the environment. In cases where modifications are common and highly variable, a plastic, experience-based mate preference may be more adaptive than a limited, innate response (Botero and Rubenstein 2012; Cotton *et al.* 2006; Miller and Svensson 2013). In insects, and especially in fruit flies, one such phenotypic trait is that of adult size (Amitin and Pitnick 2007; Credland *et al.*

1986; Pitnick and Garcia-Gonzalez 2002). Size in insects tends to be positively correlated with female fecundity (e.g. more eggs are laid by large females than by small females) as well as “attractiveness” and male competitiveness (Bonduriansky 2001). As such, larger individuals often have higher mating frequencies, and higher reproductive output compared to smaller individuals (Credland *et al.* 1986; Honek 1993; Morimoto *et al.* 2016; Partridge *et al.* 1987; Parker and Pizzari 2010; Pitnick and Garcia-Gonzalez 2002; Wigby *et al.* 2015). Behavioral phenotypic differences can also result from variation in the offspring’s developmental diet. For example, fruit flies raised on different media have been found to display distinct mating patterns (Abed-Vieillard *et al.* 2016; Dodd 1989; Najarro *et al.* 2015; Sharon *et al.* 2010). In cases where the quality of the juvenile diet differs, differences in mating strategy can be elicited, creating a situation of condition-dependence in mate choice wherein high quality individuals are more likely to show the strongest mate preference (reviewed by Cotton *et al.* 2006). For example, Hunt *et al.* (2001) demonstrated, by varying the quality of the juvenile diet, that the condition in adult female black field crickets (*Teleogryllus commodus*) was correlated with variation in preferences for males. Females that were raised on high quality diet were both larger and had higher survival rates than females raised on low quality diet, and displayed stronger directional selection for males that produced higher frequency calls (which is indicative of higher male quality). Alternatively, species may evolve a non-traditional approach to choosing mates and, instead, choose to assortatively mate with others based on their own environmentally-induced phenotype (unrelated to condition). In insects, this phenomenon has been observed in situations where there have been environmentally-induced modifications to the pheromone (e.g. epicuticular hydrocarbon; Etges *et al.* 2006; Ferveur 2005; Geiselhardt *et al.* 2012) and/or microbiome profile (Ringo *et al.* 2011). For example, male mustard leaf beetles *Phaedon cochleariae* have been

observed to prefer to mate with females that were raised on the same host plant species as a result of having a more similar cuticular hydrocarbon phenotype than those raised on a different host plant species (Geiselhardt *et al.* 2012). In *D. melanogaster*, differences in the protein availability in culture media have resulted in strong assortative mating preferences induced by changes in the hydrocarbon profile by the commensal bacteria *Lactobacillus plantarum* (Ringo *et al.* 2011). However, it is also true that dietary mediated differences in hydrocarbons may not always result in phenotypic-based sexual preference (Fedina *et al.* 2012).

Here, we set out to examine whether the quality of the developmental diet influences the mating behavior and individual reproductive success of *D. suzukii*. We studied combinations of male and female flies based on their nutritional developmental history (NDH) in both choice and in no-choice mate assays. The results of these assays revealed differences in individual choosiness (that are suggestive of condition-dependent mating) as well as differences in fecundity and offspring survival that are associated with the NDH of the parents. In addition, we show that the effects of the social context may also influence mating behavior in this species. This study shows that *D. suzukii* exhibits behavioral plasticity in mate selection and that the environment can affect phenotypic traits relevant to individual fitness – information which could be used to improve management of this invasive pest.

## **Materials & Methods**

### ***Drosophila suzukii* Population History & Culture Protocols**

In this experiment, we used *Drosophila suzukii* adults that originated from a large (~1400 adults/generation) laboratory population. This population was shared with our lab in 2014 by Dr. Justin Renkema (then of the University of Guelph, now located at the University of Florida) and

originates from individuals isolated from blackberries and raspberries collected from a Southern Ontario commercial farm during the summer of 2012 (described in Renkema *et al.* 2016) Since then, the flies have been cultured under standard laboratory conditions (25°C, 60% humidity, LD 12:12) on Rose's fly media (Rose 1984). Following a 21 day culturing cycle, on "Day 1" of the cycle, the population of flies is mixed *en masse* under light anesthesia (CO<sub>2</sub>) and transferred to a fresh set of vials containing ~10 ml of fly media, with 20-25 flies per vial. After 48 hours in these vials, the flies are transferred to a second set of fresh vials (which serves as a "back-up" stock) before being discarded 48 hours later.

### ***Experimental Diets***

To analyze the influence of different developmental environments on *D. suzukii* reproductive behavior, we raised larvae on two artificial diets in which the protein to carbohydrate ratio (P:C) was experimentally manipulated to create a high carbohydrate-low protein diet (P:C 1:3) and a low carbohydrate-high protein diet (24:1). We chose these P:C ratios based on a previous study we conducted (see Chapter 2), in which, using a nutritional geometry framework (Simpson and Raubenheimer 1993), we assessed the performance of *D. suzukii* larvae on eight different medias ranging in P:C ratio (P:C 1:12, 1:6, 1:3, 1:1, 2:1, 4:1, 8:1, 24:1). From this study we found that larval survival and eclosion rate was positively associated with the availability of protein in the media and that larval performance was significantly different across the P:C landscape. As such, the two ratios, 1:3 and 24:1, represent two very different nutritional environments for larvae. Furthermore, these two P:C ratios are likely to be similar to the environments that are encountered by *D. suzukii* in their natural habitat. For instance, the P:C ratio of ~1:3 is commonly observed in farmed and wild fresh fruit commonly attacked by *D. suzukii* in North America (Table 2.1), and the P:C ratio of 24:1 is potentially found in rotting

fruits that contain protein-rich microbial organisms (Janzen 1977). The observation that females, in a choice and no-choice scenario, lay eggs on media consisting of a 1:3 and 24:1 P:C ratio (Chapter 2) also suggests that this species potentially parasitizes both intact, fresh fruits as well as rotting fruits in the wild.

In keeping with our prior developmental study (Young *et al.* 2017; Chapter 2), the two different media were generated by way of the manipulation of the quantities of protein and carbohydrates in each recipe while keeping all other ingredients in the media constant, as outlined by Lihoreau *et al.* (2016). Specific details on the recipes used are listed in Chapter 2.

### ***Experimental Flies***

Flies with different nutritional developmental histories (NDHs) were generated by raising individuals on the two medias differing in their protein-to-carbohydrate ratio (P:C). To do this, we first collected eggs produced by flies from our lab population by placing groups of flies into half-pint laying chambers that had been outfitted with 35 mm petri dish lids (BD Falcon brand) which contained a grape-agar media (Sullivan *et al.* 2000). Approximately 18 hours later flies were removed from the half-pint chambers and eggs were sorted into groups of 20 and transferred into vials containing 10 ml of one of the 2 P:C media types. Vials were incubated under standard conditions and beginning 14 days later, adult flies were collected as they eclosed from their pupae, sorted into groups of 10 by sex under light CO<sub>2</sub> anesthesia and transferred into vials containing standard laboratory media. Virginity of females was confirmed by the examination of holding vials for the absence of hatched eggs and larvae.

### ***No-Choice Mating Assays***

In order to obtain baseline measurements of mating behavior and fitness of *D. suzukii* from different NDHs, we conducted a series of experiments in a so-called ‘no-choice’ environment. In these assays we combined (without anesthesia) a single female and single male, that had been generated and collected as virgins following the protocols described above. All four possible combinations of NDH and sex of the pairs were created (Figure S3.1), and each of these treatments consisted of 60-70 replicates each. All vials were set up for observation starting around ~9am on the 14-17th day of the fly’s life cycle. Once combined, the vials were placed horizontally on a vertical board in an evenly lit, bright, quiet room and, observed continuously for a maximum of 6 hours for the initiation and termination of copulation, which were measured to the closest second. At the end of the assay, we lightly anesthetized the flies and removed all of the males, leaving the single mated females in the vials. These vials were then incubated for ~24 hours at which time the mated female, under anesthesia, was carefully removed from the vial and, placed into an individual egg-laying chamber (Kartell Polyethylene 7ml vials 226245-10) and closed with a cap that contained grape-agar media. The number of eggs each female laid in the vial (that she was previously housed in) was counted. After ~16 hours in the incubator, the females were removed from the egg-laying chambers and the number of eggs laid by each individual female in the media counted. Immediately following counting, in cases where there were >5 eggs, half were placed into a vial containing ~10 ml of media of a 1:3 P:C ratio and the remainder into a second vial containing ~10 ml of media of a 24:1 P:C ratio. These vials were then incubated and at both 16 and 18 days post-egg transfer, the number of flies that successfully eclosed in each vial were counted.

### ***Choice Mating Assays***

To examine the impact of the opportunity for sexual selection to operate (via intra-sexual competition and/or inter-sexual choice), we conducted two experiments in which a single focal individual (raised in one of the two different nutritional environments) was placed into a chamber with members of the opposite sex (and that had also originated from different nutritional environments; Figure S3.1). We conducted one assay in which each of the vials contained a single focal male and two females (“male choice assay”) and a second in which each of the vials contained a single focal female and two males (“female choice assay”). In order to be able to identify the NDH of the fly that mated with the focal fly during the mating assay, we dusted all non-focal flies *en masse* ~16 hours prior to the mating assay with orange or pink non-toxic dry pigment (~0.01 g/60 flies; DayGlo® Color Corp. AX-12-5 Neon Red and AX-15-N Blaze Orange). Flies were dusted with the two pigments in a balanced manner between treatments (NDH), so that half of the flies from each treatment were dusted one colour and the other half dusted the other colour. Once flies were dusted, they were immediately sorted, under light anesthesia, into groups of two flies that differed both in NDH and dust colour, and placed into vials containing standard laboratory media. Focal flies (not dusted) were also transferred singly, under light anesthesia, into vials containing standard laboratory media. Dusting and/or transfer of flies into the vials was done ~16 hours before the start of the mating assay to give flies enough time to remove excess dust and recover.

For the mating assay, four different combinations of flies were created based on the focal fly NDH and the dust colour of the two potential mates (Figure S3.1) with a total of 55-72 replicates created per combination. The assay began at ~9am on the 14-17th day of the flies’ life cycle by combining the non-focal paired flies with the focal flies via lightly tapping the flies into

the vials. Flies in vials were observed and scored in the manner above and the start time and end time of each mating recorded. For each mating that was observed, we also identified the NDH of the non-focal fly chosen using a handheld UV light (Vansky UV Flashlight B011LPWXV6) which causes the pigment to fluoresce, allowing for a more efficient means of colour identification. At the end of the assay, we counted the number of eggs laid by each female and measured survivorship of the offspring following the same protocol as described in the no-choice mating assay.

### ***Statistical Analysis***

All statistical analyses were conducted using R (v 3.3.1; R Core Team 2016). Mating rates were analyzed using general linear models (GLMs) constructed with quasibinomial error distributions. In the no-choice experiment, the NDH background treatment of both flies and their interaction were the independent variables, and the occurrence/absence of mating was the dependent variable. For the two choice experiments, our model also included the specific colour-combination used to distinguish between the two competing flies (there were four possible combinations of NDH treatment and dust colour). Comparisons of the mating rates involving the four different NDH combinations obtained from the three experiments were also analyzed using General Linear Models (GLMs) constructed with quasibinomial error distributions. The significance of factors in all tests was determined using the *Anova* function (in the *car* package) using a type II sums of squares analysis of deviance test.

As mating latency was consistently non-normal (and could not be transformed to normality using either log and square root transformations), we analyzed this data using the Scheirer-Ray-Hare method (Scheirer *et al.* 1976), where the dependent variable (latency) is first ranked and then analyzed using a two-(or three-)way ANOVA (using function *aov*). We



analyzed latencies using datasets that a) contained latency values obtained from both mated flies and substituted latency values equivalent to the duration of the assay used for vials in which no mating was observed and b) only latency values from flies that were observed to start mating during the observation window. Independent variables used in these analyses of choice and no-choice assays were the same as those described above. The four different NDH combinations in the three experiments were compared using a Kruskal-Wallis test (where maximum latency values were capped across the three assays so that differences between the total length of the three trials was not confounding). Post-hoc tests, when conducted were performed using the *kruskalmc* function in the *pgirmess* package. Copulation durations in each of the choice and no-choice assays were also analyzed using the Scheirer-Raye-Hare method and, due to non-normal distributions, we used the same methods and independent described above. Comparisons of median copulation durations between the trials for each of the four different NDH combinations were also conducted using the same Kruskal-Wallis methodology.

Egg production was analyzed using data collected during the first ~16 hours post-mating, from the 16 to~32 hours post-mating period and across the whole 32 hour period using GLMs constructed with quasipoisson error distributions. For both the choice and the no-choice experiment, the NDH treatment of the mating female fly, the mating male fly and their interactions were independent variables, and the number of eggs laid was the dependent variable. Comparisons of egg production between the choice and no-choice assays for each of the four different NDH combinations were also analyzed using general linear models (GLMs) constructed with quasipoisson error distributions. The significance in all tests was determined using the *Anova* function with type II sums of squares, and a Tukey HSD test was used when necessary to locate the specific location of differences between group means.

Survivorship of the larvae on the two different medias was analyzed separately using GLMs structured with quasibinomial error distributions. For all experiments, the proportional survivorship was the dependent variable and the independent variables were the male NDH treatment, the female NDH treatment and their interaction. Comparisons of survivorship between the three choice and no-choice assays for each of the four NDH combinations were also analyzed using general linear models (GLMs) constructed with quasibinomial error distributions. The significance in all tests was determined using the *Anova* function with type II sums of squares, and a Tukey HSD test was used when necessary to locate the specific location of differences between group means.

## **Results**

### ***Mating Rates & Mate Selection***

In the no-choice assay, neither male nor female NDH had a significant effect on the likelihood that the two flies would mate during the observation period (Table 3.1a). In the female choice experiment, however, we saw that females from the 1:3 NDH treatment were more likely to mate than those from the 24:1 NDH (Table 3.1b). In the male choice experiment we observed that the number of matings increased when 1:3 females were orange and 24:1 females were pink compared to the situation in which the colours were reversed (Table 3.1c).

When the four potential combinations of NDH males and females were compared across the choice and no-choice trials we observed that in the case where 1:3 females mated with 1:3 males, more matings occurred in the male choice than in the female and no-choice experiments (Table 2a; Figure 3.1). Similarly, where 24:1 females mated with 1:3 males more matings occurred in the male choice experiment compared to the no choice experiment (Table 2d; Figure

3.1). No significant differences in mating likelihood were found between the two other NDH combinations (Table 2bc; Figure 3.1)

Our analysis of the outcome of matings of focal flies in both the female and male choice experiments did not reveal any significant biases associated with their partner's NDH (Table 3.2).

### ***Mating Latency***

Neither female nor male NDH significantly influenced the time to mating in the no choice trial when we included both the times from mated and non-mated flies (where the latency of non-mated flies was set to be equal to the maximum duration of the experiment) (Table 3.4a).

However, when we analyzed only the flies that had successfully mated during the assay we observed that 1:3 males mated later than 24:1 males (Table 3.4a). When examining the times of both mated and non-mated replicates in the choice trials, latency was significantly affected by focal fly treatment, albeit with opposing trends. Whereas 1:3 females mated sooner in the female choice assay (Table 3.4b), 1:3 males mated later in the male choice assay (Table 3.4c). No significant affects from treatment or combination of flies was found in the choice assays when only mated fly times were assessed (Table 3.4bc).

From the comparisons of the four individual NDH combinations across the choice and no-choice trials, in which both mated and non-mated fly times were included in the analyses, we found that when 1:3 males mated with 1:3 females, it took significantly longer for matings to start in the male choice assay compared to the female choice assay (but not compared to the latency in the no-choice assay; Table 3.4a). No significant differences were observed between the other three NDH combinations when both mated and non-mated fly data was analyzed together (Table 3.5a-d). In contrast, when we examined the start times of only those flies that

mated we found, mating latencies were significantly longer on average in the no-choice assays when the NDH of the sexes differed (Table 5cd; Figure 3.2) and that there was no significant differences in latencies when the NDH of the sexes were the same (Table 5ab; Figure 3.2).

### ***Copulation Duration***

The NDH of males and females did not significantly influence copulation duration length in the no-choice assay (Table 3.6a). In the female choice assay, a significant interaction between female treatment and mating duration was detected, wherein females raised on the 1:3 media mated for longer periods compared to females raised on 24:1 media. In the male choice assay, neither male nor female NDH treatment nor their interaction was found to significantly influence copulation duration (Table 3.6c).

The comparison of copulations between the choice and no-choice assays for each of the four NDH combinations revealed a significant difference in duration between the choice and no-choice assays when the sexes differed in NDH. In the case where 24:1 females mated with 1:3 males, those in the male choice assay mated for significantly longer than those in the female choice assay, but not when compared to those in the no-choice (Table 3.7c; Figure 3). In the case where 1:3 females mated with 24:1 males, those in the no-choice assay mated significantly longer than those in the choice assays (Table 3.7d; Figure 3).

### ***Egg Production***

#### *Fecundity in the first ~16 hours post-mating*

The average number of eggs that females produced in the no choice experiment was significantly greater when both males and females were from the 24:1 NDH compared to the two combinations in which females mated to males of a 1:3 NDH (Table 3.8a). Focal fly treatment

and non-focal fly combination did not appear to influence egg production in the choice assays (Table 8bc; Figure 3.4).

In the comparisons of average egg numbers for each of the four NDH combinations significant differences between the choice and no choice assays were revealed. Where females had mated to 24:1 males, a significantly greater number of eggs were laid in the male choice and no-choice assays compared to the female choice assay (Table 3.9bd) and where females had mated to 1:3 males, significantly more eggs were laid in the male-choice assay than in the female choice and no-choice assays (Table 3.9ac).

*Fecundity during hours 16 to 32 post-mating*

The average egg production in the no-choice assay revealed an interaction between male and female NDH and female egg production, as 1:3 females that mated with 1:3 males laid significantly fewer eggs than females mated to 24:1 males (but not when 24:1 females mated with 1:3 males; Table 3.10a). Focal fly treatment and non-focal fly combination did not appear to influence egg production in both of the choice assays (Table 3.10bc).

A significant difference in egg production between the choice and no-choice assays was detected in two of the four NDH combinations. In the case where both sexes shared a NDH of 1:3, pairs in the female choice assay produced more eggs than pairs in the no-choice assay (but not when compared to the male-choice assay; Table 3.11a) In the case where both sexes originated from the 24:1 media, pairs in the female choice assay produced a greater number of eggs than pairs in the male-choice assay (but not when compared to the no-choice assay; Table 3.11b).

### *Total egg production (hours 0-36, post-mating)*

The NDH of males and females had a significant effect on total egg production in the no-choice assays: pairs that shared a 24:1 NDH produced a significantly greater numbers of eggs than pairs where the female was mated to a male of a 1:3 NDH (Table 3.12a; Figure 3.4). Focal fly treatment and non-focal fly combination did not influence egg production in either of the choice assays (Table 3.12bc; Figure 3.4).

Three of the four NDH combinations differed in total egg production in different ways between the choice and no-choice assays. When both sexes were of a 1:3 background, females in the male choice assay produced significantly more eggs than females in the other two assays (Table 3.13a; Figure 3.5). When both sexes were of a 24:1 background, females in the no-choice experiment produced a greater number of eggs when compared to the other two experiments (Table 3.13b; Figure 3.5). Finally, when 1:3 females mated with 24:1 males, females in the male choice assay produced, on average significantly more eggs compared to those in the female choice assay (but not when compared to those in the no-choice assay; Table 3.13c; Figure 3.5).

### ***Egg-to-Adult Offspring Survivorship***

#### *On 24:1 media*

In the no-choice assay survivorship of eggs-to-adults on the 24:1 media did not significantly differ depending on the NDH of either males or females or their interaction (Table 3.14a). As well, focal fly treatment and non-focal fly combinations did not appear to influence survivorship on 24:1 media in both of the choice assays (Table 3.14bc).

In all four NDH combinations, comparisons between the choice assays and no-choice assay revealed significant differences between groups (Table 3.15a-d; Figure 3.6). In the two combinations where females were mated to 1:3 males, a greater fraction of larvae survived to

adulthood in the male choice assay than in the female choice assay (but not when compared to the no-choice assay; Table 3.15ac; Figure 3.6). When females were mated to 24:1 males, offspring fared significantly better in both the no-choice assay and male choice assay when compared to the female choice assay (Table 3.15bd; Figure 3.6).

#### *Survivorship on 1:3 media*

Although our GLM indicated a significant interaction between survivorship and NDH of males and females on 1:3 media in the no choice assay ( $p=0.0028$ ), the subsequent post-hoc Tukey test did not detect any statistically significant difference between groups (at the  $\alpha=0.05$  family-wise level). Focal fly treatment and non-focal fly combination did not appear to influence survivorship on 1:3 media in either of the choice assays (Table 3.15bc).

The NDH combination of mated pairs did not influence survivorship on the 1:3 media (Table 3.16a-d). While our GLM revealed a significant interaction when 24:1 females mated with 1:3 males ( $p=0.0002$ ; Table 3.16c), when we followed up with a post-hoc Tukey test we did not identify any statistically significant difference between groups (at the  $\alpha=0.05$  family-wise level).

## **Discussion**

*Drosophila suzukii* is a highly successful invasive pest species that threatens soft fruit crops worldwide (Bolda *et al.* 2010). In this study, we investigated two largely unknown aspects of their ecology and developmental biology. First, we explored whether nutritional developmental history (NDH) influenced sexual behaviour in this species. Overall, we found that flies that had developed on the lower quality media (1:3 proteins : carbohydrates) were both less attractive and less choosy compared to flies that had developed on the higher quality media (24:1). As such,

low-quality flies in this species may assortatively mate, although not necessarily by design. We also found evidence that either females make sub-optimal choices when selecting mates or that individuals vary their reproductive investment based on their current social circumstances. In the second part of this study, we examined the relationship between mate choice and individual fitness variation in *D. suzukii*. Here we found that the NDH had an impact on both egg production and survival and that this impact was also highly influenced by social context, suggesting that choice is important to the fitness of this species. Together, these results provide valuable information on the biology of *D. suzukii* which can be incorporated into future pest management plans.

#### ***D. suzukii* exhibit nutritionally-induced phenotypes relevant to sexual selection and fitness**

In all three of our experiments, adult *D. suzukii* showed differences in mean mating behaviors that were associated with their NDH, which suggests individual behavioural phenotypes exhibit phenotypic plasticity with respect to developmental nutrition characteristics. Although the flies did not show a bias for types of mates based on their NDH, we found evidence that males which had developed on the low quality 1:3 media were generally less sexually attractive to females that had developed on the better quality 24:1 diet. This can be seen from both the later start times and the shorter mating durations in the choice trials compared to the no-choice trials where a 1:3 male mated with a 24:1 female. In *D. melanogaster* both copulation latency (Ratterman *et al.* 2014, as well as Tennant *et al.* 2014) and copulation duration (Bretman *et al.* 2013) are under joint control of both sexes, with females mating less readily and more briefly with less attractive males. The observation that the 1:3 males were apparently less attractive to 24:1 females is not too surprising considering that, in our previous nutritional geometry experiment with *D. suzukii*



(Chapter 2), we observed, on average, longer egg-to-adult developmental times and smaller adult weights when flies developed on the poorer quality (low protein) medias. A similar result was obtained by Silva-Soares *et al.* (2017) in an experiment that used a nutritional geometric methodology. We also observed that, in the male choice experiment, the combination of dust colour and the NDH of the potential mates had an effect on mating rate, with the number of matings increased when 1:3 females were dusted orange and 24:1 females were dusted pink. However, since the male flies did not show mate preferences based on NDH, it is unlikely that this bias had a significant impact on mate choice. In line with the observation that 1:3 females appear to be less choosy about who they mate with, it may be that the orange dust made females easier to locate for males, which might explain this trend. Among insects, it is well documented that the nutritional composition of the diet can have important consequences for developmental pathways (Andersen *et al.* 2009; Carrel & Tanner 2002; Lihoreau *et al.* 2016; Maklakov *et al.* 2008; May *et al.* 2015; Morimoto & Wigby 2016; Raubenheimer & Simpson 2003; Rodrigues *et al.* 2016) and directly influence traits associated with reproductive success. Slower developmental times and smaller average adult sizes (e.g., Thomas 1993) are two phenotypic characteristics often observed in insects that have developed on a lower quality diet, and which are also correlated with lower reproductive potential (Amitin and Pitnick 2007; Bonduriansky 2001; Credland *et al.* 1986; Nijhout *et al.* 2013; Pitnick and Garcia-Gonzalez 2002). In the no-choice assay, we observed that egg production differed between NDH treatments: when females were mated to a 1:3 male they experienced reduced egg production for both the first ~16h, the second 26h post-mating and in total. However, these differences were not observed when males and females had the ability to choose between two potential mates. We hypothesize that egg production was lower in the no-choice vs. choice because the social context either has a large

influence on a) the reproductive investment between certain phenotypes or b) the reproductive potential between certain phenotypes (discussed below). No significant differences were observed in offspring survivorship in terms of the NDH combination of the mated pairs in any of the three assays. The results on survivorship, however, should be treated with some caution as each replicate (individual female) was represented by a very small number of surviving offspring (average of 0.86 per female or 13%), numbers which are much lower than what was expected based on the 50% mortality on the low quality diet that we had previously observed (Chapter 2). One possible reason for the low larval survivorship in our experiments could be that the very low numbers of larvae in the vials (average of 3.45 eggs) hindered the process of larval “working” or softening the hard agar-based media (reviewed by Ashburner *et al.* 2005). This process may be important for accessing nutrients. In addition to differences in attractiveness and/or vigor, we also observed a difference in the degree of choosiness exhibited by flies based on their NDH. Flies from the 1:3 NDH tended to be less “choosy” than flies of a 24:1 NDH. This trend was particularly obvious in the female choice assay, where, in comparison to the 24:1 females, 1:3 females mated significantly sooner and in a greater proportion, suggesting that 1:3 females are more willing to mate as well as forgo a more thorough assessment of male quality before deciding to mate. If 1:3 females are, indeed, of lower quality, then a condition-dependent strategy could provide a number of fitness advantages. If the process of discriminating between potential mates is costly (e.g., time spent sampling takes away from time spent foraging and ovipositing), then lower quality flies that are less able to pay the costs of being choosy could potentially gain greater fitness benefits by being less choosy about who they mate with (Cotton *et al.* 2006). As seen in our fecundity analysis, it appears that, in *D. suzukii*, this may be also context-dependent, as 1:3 flies appeared to incur fitness losses more when they did not have a

choice of mates. We discuss this point, and its implications for management, in this species below.

### **Potential for assortative mating among low quality individuals in *D. sukuii***

While we did not observe assortative mating based on NDH in the three different assays, the combination of males being both less attractive and females less choosy when developing on low quality media could potentially result in low quality flies assortatively mating. Furthermore, since 1:3 males mated more with 1:3 females in the male choice assay, they may also prefer females from the same NDH, this increases the likelihood of assortative mating arising in certain situations (*e.g.*, in social environments where males are less common than females). The other (not exclusive) possibility for why 1:3 males mated more frequently with 1:3 females could be because 1:3 females were also less choosy in who they mated with compared to the 24:1 females. Assortative mating is a relatively common phenomenon in sexually reproducing species and has the potential to result in greater local adaptation (Bateson 1983). From our fecundity and larval survival analysis, this does not appear to be the case between 1:3 flies, at least in the no-choice assay, because the total number of eggs produced from matings between 1:3 males and 1:3 females was significantly fewer compared to the number of eggs produced from matings between 1:3 flies and 24:1 flies. However, it is possible that any benefit gained from assortatively mating may have been offset by the direct costs of being of a lower quality. Although we did not observe statistically significant evidence of assortative mating between the 24:1 flies, it does appear, at least in the no-choice assay, that matings between these two phenotypes did have benefits to their fecundity.

## **Females appear to make poor mate choices and/or flies vary their reproductive investment based on their social context**

Our analysis of fecundity and offspring survival in relation to the NDH combination revealed that *D. suzukii* females experienced a reduction in total fecundity and offspring survival when they had a choice of mates. One possible explanation for this could be that, simply put, female *D. suzukii* make poor mate choices. Poor mate choice is not uncommon among insects and particularly among *Drosophila* species (Parker 1979; Gowaty 1997; Gowaty & Buschhaus 1998; Holland & Rice 1998; Gavrilets *et al.* 2001). Rather than provide an honest signal of quality, male ornaments may actually manipulate or coerce females into choosing them at the advantage of the males' fitness but to the detriment of the females' fitness (Arnqvist 2006). Sperm is relatively much less costly to produce compared to eggs, and, thus, a sexual conflict can ensue when males gain more benefit from mating with many individuals over a few high quality individuals, evolving dishonest strategies to accomplish this (Kokko *et al.* 2003). It should be noted, however, that females may gain fitness benefits in other ways not measured in our experiments (e.g., indirect fitness, manifested as offspring of superior reproductive success) and which requires further investigation in future studies. Alternatively, another possibility for lower egg numbers and reduced offspring survival when females choose mates is that females may allocate their reproductive investments differently based on the current social context. In the female choice trial, for example, females did not have to compete for male attention and also had more than one male available to sire her offspring. As such, females may not have experienced a competitive pressure to invest into producing lots of offspring sired from one male and instead preferred to invest more heavily into producing moderate numbers of offspring of mixed paternity. In other words, females were choosing to 'share' eggs among multiple fathers instead

of giving one male a large portion of eggs for fertilization. The benefits of a mixed paternity are numerous and include an increase in genetic diversity and fertilization success and, where pre-copulatory mate-choice cues are unreliable or absent, polyandry in females may even be selected for (reviewed by Jennions and Petrie 2000). Interestingly, we did find some potential (and admittedly highly speculative) evidence that females in the choice experiment may have been intending to ‘share’ their brood with the two males present and invest less per mating (so called “bet-hedging”, see Garcia-Gonzalez *et al.* 2015). For example, 24:1 females mated for significantly shorter periods of time with males in the female choice assays than with the males in the male choice assay. This was somewhat surprising as in *D. melanogaster* there is strong pattern of *longer* copulation durations when multiple males are present (Friberg 2006 and Bretman *et al.* 2010) presumably in response to the greater risk of sperm competition. This may represent female *D. suzukii* taking an active role in early termination of copulations (Bretman *et al.* 2013). Although no significant difference in egg production was observed in the 24:1 females between the female and male choice trials, survival of the offspring was significantly worse on the 24:1 media when females had a choice of more than one mate. Furthermore, 1:3 females laid more eggs in total when they did not have a choice of mates with more of those offspring surviving on the 24:1 media. Similarly males may also be bet-hedging by attempting to mate with multiple females rather than ‘place all of their eggs into the same basket’, so to speak (e.g, in the male choice assay; see Pitnick & Markow 1994). We did not find evidence of this, in our experimental results, but this may be due to the fact that males do not need to reduce copulation durations between females in order to provide an equal ‘share’ of sperm since it is likely to be much more plentiful (at least in our experimental context). Although we did not deliberately set out to measure if flies mated with both flies in the vial or not, we did observe 19-30% of the

males mating with the second female in the male choice trials. Alternatively, it is also possible that, instead of females allocating fewer progeny to each mate (in the female choice assay), females may instead be allocating more progeny to the one available male sire (in the male choice assay). The occurrence of female-female competition has been found to influence the intensity of sexual selection in many species (Kvarnemo and Ahnesjo 1996), which may also influence parental investment. As before, the same may be said for the males (Kvarnemo and Ahnesjo 1996).

### **Opportunity for mate choice and its consequences for *D. sukukii* mating and fitness**

In our studies with *D. sukukii* we found that the ability to choose mates resulted in differences between treatments in both mating behavior and individual fitnesses. When females chose to mate with a (low-quality) 1:3 male, the total number of eggs females produced, on average, was significantly greater compared to when females were ‘forced’ (in the no-choice environment) to mate with a 1:3 male. Whether this was because females were a) able to avoid deleterious fitness consequences from mating with a poor-quality male in the choice environment or because b) females simply invested less into egg production due to lower levels of male stimulation in the no-choice assay, is a question that remains unanswered. Either way, this suggests that the social circumstances potentially have a significant influence on the fitness of individual *D. sukukii* when the paternity is from a male of a low-quality phenotype. Mate choice has been found to be highly beneficial for maximizing fitness (Kokko *et al.* 2003; Jennions and Petrie 2000). For instance, larvae exhibit better competitive success when they came from parents who were given the opportunity to choose mates (Partridge 1980; Long *et al.* 2012). Females have also been documented to invest in offspring in accordance to the perceived

attractiveness of their mate, with greater attractiveness of mates receiving greater parental investment (Horvathova *et al.* 2012; Bateson 1983). As discussed above, we also found that, in choice situations, females appear to make poor mating decisions and/or flies invest reproductive efforts differently based on their social context. This was demonstrated by the fewer total number of eggs laid and the poorer survivorship of offspring in the female choice trials compared to the no-choice trials. Our experiments also reveal that the context can potentially result in differences in offspring production. When we compared total progeny among the apparently choosier, more attractive, higher quality 24:1 phenotypes, assortative pairing surprisingly resulted in fewer total progeny in both the female and male choice assays compared to the no-choice assay. This potentially suggests that higher quality individuals may either invest reproductive efforts more moderately in choice situations where there are more potential mating opportunities or make poorer choices (e.g., higher chance of being attracted to dishonest cues about mate quality), and is an interesting subject for future research.

### **Consequences of *D. sukuzii* mate choice and fitness: an interaction between phenotype and social context**

The differences in *D. sukuzii* mating behavior and fitness, between and within the three different experimental assays, suggest that population growth rate in this species potentially depends on the interaction between the types of adult phenotypes present (high or low quality) and the current social dynamics (no-choice, male choice, female choice) at any given time. This could have large implications for the management of this pest as different habitats may experience different growth rates. If we consider populations consisting of both low and high quality phenotypes, and in which potential mates and competitors are scarce (e.g. as in a very

small population), the NDH of the flies is likely to have the greatest impact on total egg production, with females that mated to low quality males experiencing the greatest loss. Thus in populations consisting of only one phenotype, those consisting of all high quality individuals would be most likely to produce a significantly greater number of offspring. In the situations where there are mixed phenotypes of differing quality and potential mates and competitors are both abundant (e.g., as in a large population), NDH may become less of a factor in terms of fitness variation and, instead, female choice may play a more considerable role in determining population growth. In the case where females hold more “power” over who they mate with, populations of this social construct may experience a more moderate type of growth rate as we found females to make poorer mate choices and/or invest less generously in the number and quality of offspring. However, the effect of female competition on reproductive investment should still be considered in these populations as it may counteract the reduced egg production from poor female choice/mating investment. Ultimately more research is needed to fully understand how females and males invest reproductive efforts in multi-female-multi-male social constructs. In contrast, where males exhibit mate choice, populations with this social construct may experience greater growth rates (relatively), as males were found to make more adaptive mate choices. The same may be true in populations where there is a female sex-bias, due to males potentially making better mate choices and/or females investing more generously in the number and quality of offspring they sired per male. Finally, a population of mixed phenotypes that is highly male-biased may experience a relatively moderate increase in offspring numbers based on the fact that although males were found to potentially make better mate choices, these benefits may be offset by females investing less reproductively per mating as a strategy to increase mixed-paternity.



There are a number of important lessons that can be drawn from the results of our assays when considering future plans for the control of this pest species. First of all, the fact that *D. suzukii* exhibits phenotypic plasticity with consequences to sexual selection and fitness implies that population growth rate potentially varies between habitat types. For example, areas where crops and/or surrounding hosts (e.g., wild fruits) are high in carbohydrates and low in protein (pre and post egg-transfer; Chapter 2) may experience lower population growth rates due to reduced fecundity. Furthermore, the promotion of mixing “low quality” flies with high quality flies could help reduce population growth rates. Secondly, our results give support to the use of the sterile insect technique (Klassen and Curtis 2005) to control *D. suzukii*. Since releasing large numbers of males in the environment could, potentially, cause females to adopt the ‘mixed-paternity strategy’ described earlier, this strategy may not only result in a reduction in egg production due to mating with infertile flies but also result in a reduction in egg production due to females and/or males moderating their reproductive investment.

## **Conclusions**

The results of our study provide preliminary evidence of nutritionally-associated phenotypic plasticity in *D. suzukii* and that phenotypes of adults that developed on protein-poor/carbohydrate-rich diets tend to be less reproductively successful as adult individuals. Male flies that developed on lower quality media were not only less attractive to potential mates but they also imparted negative fitness consequences to the females that mated with them in no-choice environments. Females raised on lower quality media were also less choosy when it came to choosing mates, potentially indicative of a ‘condition-dependent’ mating strategy, which could also result in ‘inadvertent’ assortative mating between male and female low-quality flies.

Assortative mating could have negative implications to female fitness in situations where a choice of males is not typical. Integrating these trends reveals that mate choice in *D. suzukii* depends, potentially, on the combination of the phenotypes and the social construct within the local vicinity at any given time. Furthermore, the choice of mates, in itself, has important fitness consequences that can be both beneficial and deleterious depending on the phenotype combination of mating pairs. If we wish to obtain a more thorough depiction of mating behavior in this species, future research will also need to look at behaviors and fitness of individuals in multi-female-multi-male contexts and through the measurements of other potential indirect and direct benefits not measured here.

## References

- Abed-Vieillard, D., Cortot, J., Everaerts, C. & Ferveur, J-F. 2013. Choice alters *drosophila* oviposition site preference on menthol. *Biol Open*, 3: 22-28. doi 10.1242/bio.2013673
- Amitin, E. G. & Pitnick, S. 2007. Influence of developmental environment on male- and female-mediated sperm precedence in *Drosophila melanogaster*. *J Evol Biol*, 20: 381–391, doi:10.1111/J.1420-9101.2006.01184.X
- Anderson, P., & Anton, S. 2014. Experience-based modulation of behavioural responses to plant volatiles and other sensory cues in insect herbivores. *Plant Cell Environ*, 37: 1826-1835.
- Arnqvist, G. 2006. Sensory Exploitation and Sexual Conflict. *Philos Trans R Soc Lond B Biol Sci*. 361: 375–386.
- Ashburner M., Golic K.G., & Hawley, R.S. 2005. 2nd edn. Cold Spring Harbor Laboratory Press; Cold Spring Harbor, NY: 2005. *Drosophila: a laboratory handbook*.
- Awmack, C.S., & Leather, S.R. 2002. Host Plant Quality and Fecundity in Herbivorous Insects. *Annu Rev Entomol*. 47: 817-844.
- Bateson, P. 1983. *Mate Choice*. Cambridge University Press.
- Begon, M. 1986. Yeasts and *Drosophila*. *The Genetics and Biology of Drosophila*, Vol. 3b (ed. by M.Ashburner, H.Carson and J. N.Thompson), 345–383. Academic Press, London.
- Bellamy., E. B., Sisterson., M. S., & S. S. Walse. 2013. Quantifying Host Potentials: Indexing Postharvest Fresh Fruits for Spotted Wing *Drosophila*, *Drosophila suzukii*. *PLoS ONE*, 8(4): e61227. doi:10.1371/journal.pone.0061227.
- Bolda., M. P., Goodhue., R. E., & F. G. Zalom. 2010. Spotted wing drosophila: potential economic impact of a newly established pest. Giannini Foundation of Agricultural Economics, University of California.
- Bonduriansky, R. 2001. The evolution of male mate choice in insects: a synthesis of ideas and evidence. *Biol Rev Camb Philos Soc*, 76: 305–339.
- Botero, C.A., & Rubenstein, D.R. 2012. Fluctuating Environments, Sexual Selection and the Evolution of Flexible Mate Choice in Birds. *PLoS ONE*, 7(2): e32311. doi.org/10.1371/journal.pone.0032311
- Bretman, A., Wetmancoat, J.D., & Chapman, T. 2013. Male control of mating duration following exposure to rivals in fruitflies. *J Insect Physiol*, 59: 824-827. doi.org/10.1016/j.jinsphys.2013.05.011

Bretman, A., Fricke, C., Hetherington, P., Stone, R. & Chapman T. 2010. Exposure to rivals and plastic responses to sperm competition in *Drosophila melanogaster*. *Behav Ecol*, 21: 317-321. doi.org/10.1093/beheco/arp189

Carrel, J. E., & E. M. Tanner. 2002. Sex-specific food preferences in the Madagascar hissing cockroach *Gromphadorhina portentosa* (Dictyoptera: Blaberidae). *J Insect Behav*, 15(5): 707-714. doi:10.1023/A:1020704108399.

Chaine AS, & Lyon BE. 2008. Adaptive plasticity in female mate choice dampens sexual selection on male ornaments in the lark bunting. *Science*, 319: 459–462.

Cockburn, A., Osmond, H.L., & Double, M.C. 2008. Swingin'in the rain: condition dependence and sexual selection in a capricious world. *Proc R Soc Lond Ser B Biol sci*, 275: 605-612.

Cotton, S., Small, J., & Pomiankowski, A. 2006. Sexual selection and condition-dependent mate preferences. *Curr Biol*, 16: 755-765.

Credland, P. F., Dick, K. M. & Wright, A. W. 1986. Relationships between larval density, adult size and egg production in the cowpea seed beetle. *Callosobruchus maculatus*. *Ecol Entomol*, 11: 41–50, doi:10.1111/j.1365-2311.1986.tb00278.x.

Cooper., D. M. 1959. Food preferences of larval and adult *Drosophila*. *Evolution*, 14: 41–55.

Diepenbrock, L. M., & H. J. Burrack. 2017. Variation of within-crop microhabitat use by *Drosophila suzukii* (Diptera: Drosophilidae) in blackberry. *J Appl Entomol* 141: 1-7.

Dodd, D.M.B. 1989. Reproductive isolation as a consequence of adaptive divergence in *Drosophila pseudoobscura*. *Evolution*, 43(6): 1308-1311.

Etges, W.J., Veenstra, C.L. & Jackson, L.L., 2006. Premating isolation is determined by larval rearing substrates in cactophilic *Drosophila mojavensis*. VII. Effects of larval dietary fatty acids on adult epicuticular hydrocarbons. *J Chem Ecol*, 32: 2629-2646.

Ewing, A.W. 1983. Functional aspects of *Drosophila* courtship. *Biol Rev*, 58: 275-292.

Fedina, T. Y., Kuo, T. H., Dreisewerd, K., Dierick, H. A., Yew, J. Y., & Pletcher, S. D. 2012. Dietary effects on cuticular hydrocarbons and sexual attractiveness in *Drosophila*. *PLoS ONE*, 7:e49799.

Ferveur, J-F. 2005. Cuticular hydrocarbons: Their evolution and roles in *Drosophila* pheromonal communication. *Behav Genet*, 35(3):279-295

Friberg, U. 2006. Male perception of female mating status: its effect on copulation duration, sperm defence and female fitness. *Anim Behav*, 72: 1259-1268.

Garcia-Gonzalez, Francisco, Yukio Yasui, & Jonathan P. Evans. 2015. Mating Portfolios: Bet-Hedging, Sexual Selection and Female Multiple Mating. *Proc R Soc Lond Ser B Biol sci*, 282: 20141525.

Gardner, D.S., Ozanne, S.E., & Sinclair, K.D. 2009. Effect of the early-life nutritional environment on fecundity and fertility in mammals. *Phil Trans R Soc B*, 364: 3419-3427. doi:10.1098/rstb.2009.0121

Gavrilets, S., Arnqvist, G. & Friberg, U. 2001 The evolution of female mate choice by sexual conflict. *Proc R Soc Lond*, B 268, 531–539. doi:10.1098/rspb.2000.1382.

Geiselhardt, S., Otte, T. & Hilker, M. 2012. Looking for a similar partner: host plants shape mating preferences of herbivorous insects by altering their contact pheromones. *Ecol Lett*, 15: 971-977.

Gowaty, P. A. 1997 Sexual dialectics, sexual selection, and variation in reproductive behavior. In *Feminism and evolutionary biology* (ed. P. A. Gowaty), 351–384. New York: Chapman & Hall.

Gowaty, P. A. & Buschhaus, N. 1998 Ultimate causation of aggressive and forced copulation in birds: female resistance, the CODE hypothesis, and social monogamy. *Am Zool*, 38: 207–225.

Head, M.L., Hunt, J., Jennions, M.D., & Brooks, R. 2005. The indirect benefits of mating with attractive males outweigh the direct costs. *PLoS Biol*, 3: 289–294.

Heimpel, G.E, Frelich, L.E, Landis, D.A., Hopper, K.R, Hoelmer, K.A., Sezen, Z., Asplen, M.K. & Wu, K. 2010. European buckthorn and asian soybean aphid as components of an extensive invasional meltdown in North America. *Biol Invasions*, 12:2913–2931

Hingle, A., Fowler, K., & Pomiankowski, A. 2001. The effect of transient food stress on female mate preference in the stalked fly *Cyrtodiopsis dalmanni*. *Proc R Soc Lond B*, 268:1239–1244.

Holland, B. & Rice, W.R., 1998. Perspective: chase-away sexual selection: antagonistic seduction versus resistance. *Evolution*, 52:1-7.

Honek, A. 1993. Intraspecific Variation in Body Size and Fecundity in Insects - a General Relationship. *Oikos* 66, 483–492, doi:10.2307/3544943

Horváthová, T., Nakagawa, S., & Uller, T. 2012. Strategic female reproductive investment in response to male attractiveness in birds. *Proc R Soc B*, 279: 163-170.

- Hunt, J., Brooks, R., & Jennions, M.D. 2005. Female mate choice as a condition-dependent life-history trait. *Am Nat*, 166: 78–92.
- Jennions, M.D. & Petrie, M. 2000. Why do females mate multiply? A review of the genetic benefits. *Biol Rev*, 75: 21-64.
- Klassen, W., & C. F. Curtis. 2005. History of the sterile insect technique. *Sterile Insect Technique*. Springer Netherlands, 3-36.
- Kokko, H., Brooks, R., Jennions, M.D., & Morley, J. 2003. The evolution of mate choice and mating biases. *Proc R Soc Lond B*, 270: 653-664.
- Kvarnemo, C., & Ahnesjo, I. 1996. The dynamics of operational sex ratios and competition for mates. *Trends Ecol & Evol*, 11: 404-408.
- Lihoreau, M., Poissonnier, L., Isabel, G., & A. Dussutour. 2016. Drosophila females trade off good nutrition with high quality oviposition sites when choosing foods. *J Exp Biol*, 219(16): 2514-24. doi:10.1242/jeb.142257.
- Lee, J. C., Bruck, D. J., Curry, H., Edwards, D., Haviland, D. R., Steenwyk, R. A., & B. M. Yorgey. 2011. The susceptibility of small fruits and cherries to the spotted-wing drosophila, *Drosophila suzukii*. *Pest Manag Sci*, 67: 1358-1367.
- Long, T.A., Agrawal, A.F., & Rowe, L. 2012. The effect of sexual selection on offspring fitness depends on the nature of genetic variation. *Curr Biol*, 22: 204-208. doi: 10.1016/j.cub.2011.12.020.
- Lushchak, O. V., Gospodaryov, D. V., Rovenko, B. M., Yurkevych, I. S., Perkhulyn, N. V., & Lushchak, V. I. 2013. Specific dietary carbohydrates differentially influence the life span and fecundity of *Drosophila melanogaster*. *J Gerontol A Biol Sci Med Sci*, 69(1): 3-12.
- Maklakov, A. A., Simpson, S. J., Zajitschek, F., Hall, M. D., Dessmann, J., Clissold, F., Raubenheimer, D., Bonduriansky, R., & R. C. Brooks. 2008. Sex specific fitness effects of nutrient intake on reproduction and lifespan. *Curr Biol*, 18: 1062-1066. doi:10.1016/j.cub.2008.06.059.
- May, C. M., Doroszuk, A., & B. J. Zwaan. 2015. The effect of developmental nutrition on life span and fecundity depends on the adult reproductive environment in *Drosophila melanogaster*. *Ecol Evol*, 5(6):1156-1168. doi: 10.1002/ece3.1389.
- Mazzi, D. 2004. Parasites make male pipefish careless. *J Evol Biol*, 17, 519–527.
- Mazzoni, V., Gianfranco, A., & Virant-Doberlet, M. 2013. Substrate vibrations during courtship in three *Drosophila* species. *PLoS ONE*, 8.

Miller, Christine W., & Erik I. Svensson. 2014. Sexual selection in complex environments. *Annu Rev Entomol* 59.

Morimoto, J., Pizzari, T. & Wigby, S. 2016. Developmental environment effects on sexual selection in male and female *Drosophila melanogaster*. *PLoS One* **11**, e0154468, doi:10.1371/journal.pone.0154468.

Morimoto., J., & S. Wigby. 2016. Differential effects of male nutrient balance on pre- and post-copulatory traits, and consequences for female reproduction in *Drosophila melanogaster*. *Sci Rep*, 6: 27673. doi:10.1038/srep27673.

Najarro, M.A., Sumethasorn, M., Lamoureux, A. & Turner, T.L. 2015. Choosing mates based on the diet of your ancestors: replication of non-genetic assortative mating in *drosophila melanogaster*. *Peer Journal*, **3**:e1173.

Padilla, D.K. & Adolph, S.C., 1996. Plastic inducible morphologies are not always adaptive: the importance of time delays in a stochastic environment. *Evol Ecol*, 10(1): 105-117.

Parker, G. A. 1979. Sexual selection and sexual conflict. In *Sexual selection and reproductive competition in insects* (ed. M.S.Blum & N. A. Blum), 123–166. New York: Academic.

Parker, T.H. 2003. Genetic benefits of mate choice separated from differential maternal investment in red junglefowl (*Gallus gallus*). *Evolution*, 57: 2157–2165.

Patrick J. Weatherhead , & Raleigh J.Robertson , In Defense of the "Sexy Son" Hypothesis. *The Amer Nat*, 117: 349-356.

Partridge, L., Ewing, A. & Chandler, A. 1987. Male size and mating success in *Drosophila melanogaster* - the roles of male and female behavior. *Anim Behav*, **35**, 555–562, doi:10.1016/S0003-3472(87)80281-6.

Petrie, M.1994. Improved growth and survival of offspring of peacocks with more elaborate trains. *Nature*, 371: 598–599.

Pitnick, S. & Garcia-Gonzalez, F. 2002. Harm to females increases with male body size in *Drosophila melanogaster*. *Proc R Soc Lond [Biol]*, **269**, 1821–1828, doi:10.1098/Rspb.2002.2090.

Promislow, D.E.L., Smith, E.A., and Pearse, L. 1998. Adult fitness consequences of sexual selection in *Drosophila melanogaster*. *Proc Natl Acad Sci USA*, 95: 10687-10692.

Ratterman, N. L., Rosenthal, G. G., Carney, G. E., & Jones, A. G. 2014. Genetic variation and covariation in male attractiveness and female mating preferences in *Drosophila melanogaster*. *G3: Genes, Genom, Genet*,4(1): 79-88.

Raubenheimer., D., & S. J. Simpson. 2003. Nutrient balancing in grasshoppers: behavioural and physiological correlates of dietary breadth. *J Exp Biol.* 206: 1669-1681. doi:10.1242/jeb.00336.

Renkema, J. M., Wright, D., Buitenhuis, R., & Hallett, R. H. 2016. Plant essential oils and potassium metabisulfite as repellents for *Drosophila suzukii* (Diptera: Drosophilidae). *Sci Rep*, 6: 21432.

Revadi, S., Lebreton, S., Witzgall, P., Anfora, G., Dekker, T. & Becher, P.G., 2015. Sexual behavior of *Drosophila suzukii*. *Insects*, 6: 183-196 doi 10.3390/insects6010183

Reynolds, J.D., & Gross, M.R. 1992. Female mate preference enhances offspring growth and reproduction in a fish, *Poecilia reticulata*. *Proc R Soc Lond [Biol]*, 250: 57–62.

Ringo, J., Sharon, G., & Segal, D. 2011. Bacteria-induced sexual isolation in *Drosophila*. *Fly*, 5(4): 310-315.

Rodrigues., M. A., Martins., N. E., Balance., L. F., Broom., L. N., Dias., A. J. S., Fernandes., A. S. D., Rodrigues., F., Sucena., E., & C. K. Mirth. 2016. *Drosophila melanogaster* larvae make nutritional choices that minimize developmental time. *J Insect Physiol*, 81: 69-80.

Ryan, K.K., & Altmann, J. 200. Selection for male choice based primarily on mate compatibility in the old field mouse, *Peromyscus polionotus rhoadsi*. *Behav Ecol and Sociobiol*, 50: 436–440.

Scheirer, C. J., Ray, W. S., & Hare, N. 1976. The analysis of ranked data derived from completely randomized factorial designs. *Biometrics*, 429-434.

Schultzhaus, J.N., Nixon, J.J., Duran, J.A. & Carney, G.E. 2017. Diet alters *Drosophila melanogaster* mate preference and attractiveness. *Anim Behav*, 123: 317-327.

Pitnick, S., & Markow, T.A. 1994. Male Gametic Strategies: Sperm Size, Testes Size, and the Allocation of Ejaculate Among Successive Mates by the Sperm-Limited Fly *Drosophila pachea* and Its Relatives," *Amer Natur*, 143: 785-819.

Silva-Soares, N. F., Nogueira-Alves, A., Beldade, P., & Mirth, C. K. 2017. Adaptation to new nutritional environments: larval performance, foraging decisions, and adult oviposition choices in *Drosophila suzukii*. *BMC ecology*, 17(1): 21.

Simpson., S. J., & D. Raubenheimer. 1993. A multi-level analysis of feeding behavior: the geometry of nutritional decisions. *Phil Trans R Soc Lond B.* 342: 381-402.

Sharon, G., Segal, D., Ringo, J.M., Hefetz, A., Zilber-rosenberg, I., Rosenberg, E. & Collier, R.J. 2010. Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. *PNAS*, 107: 20051-20056.



Simmons, L.W. 1987. Female choice contributes to offspring fitness in the field cricket, *Gryllus bimaculatus* (De Geer). *Behav Ecol Sociobiol*, 21: 313–321.

Sullivan, W., Ashburner, M., & Hawley, R.S. 2000. *Drosophila Protocols*. CSHL Press, 2000.

Tennant, HME, Sonser, E., & Long TAF. 2014. Hemiclonal analysis of interacting phenotypes in male and female *Drosophila melanogaster*. *BMC Evol Biol*, 14: 95.

Thomas H. T. 1993. Ecology of body size in *Drosophila buzzatii*: untangling the effects of temperature and nutrition. *Ecol Entomol*, 18: 84-90. doi: 10.1111/j.1365-2311.1993.tb01084.

Tscharntke, T., & R. Brandl. 2004. Plant-insect interactions in fragmented landscapes. *Annu Rev Entomol*, 49, 405–430.

Walsh, D.B., Bolda, M.P., Goodhue, R.E., Dreves, A.J., Lee, J., Bruck, D.J., Walton, Y.M., O'neal, S.D. & Zalom, F.G. 2011. *Drosophila suzukii* (diptera: drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential. *JIPM*, 2. doi 10.1603/ipm10010

Wigby, S., Perry, J. C., Kim, Y. H. & Sirot, L. K. 2015. Developmental environment mediates male seminal protein investment in *Drosophila melanogaster*. *Funct Ecol*, 30: 410–419.

## Tables & Figures

**Table 3.1.** Analysis conducted on mating rates in the no choice (a), female choice (b), and male choice (c) trials. Analysis was conducted using GLMs with quasibinomial distributions.

<b>TRIAL</b>	<b>FACTORS</b>	<b>LR<math>\chi^2</math></b>	<b>df</b>	<b>p</b>
<b>a) No choice</b>	Male treatment	0.1772	1	0.6738
	Female treatment	0.4294	1	0.5123
	Male treatment: Female treatment	0.0990	1	0.7531
<b>b) Female choice</b>	Male combination	0.3587	1	0.5493
	Female treatment	5.5863	1	<b>0.0181</b>
	Male combination: Female treatment	2.3695	1	0.1237
<b>c) Male choice</b>	Female combination	9.4927	1	<b>0.0021</b>
	Male treatment	1.8215	1	0.1771
	Female combination: Male treatment	0.7129	1	0.3985

**Table 3.2.** Comparisons in mating rates between the four NDH combinations across the three (no choice (NC), female choice (FC) and male choice (MC)) trials (a-d). Analysis was conducted using GLMs with quasipoisson distributions.

<b>TRIAL</b>	<b>LR<math>\chi^2</math></b>	<b>df</b>	<b>p</b>	<b>NC</b>	<b>MC</b>	<b>FC</b>
<b>a) 1:3 male + 1:3 female</b>	24.089	2	<b>5.878x10<sup>-6</sup></b>	<b>b</b>	<b>a</b>	<b>b</b>
<b>b) 24:1 male + 24:1 female</b>	3.5878	2	0.1663	na	na	na
<b>c) 1:3 male + 24:1 female</b>	6.5359	2	<b>0.0381</b>	<b>b</b>	<b>a</b>	<b>ab</b>
<b>d) 24:1 male + 1:3 female</b>	7.1685	2	<b>0.0278</b>	<b>a</b>	<b>a</b>	<b>a</b>

**Table 3.3.** Analysis conducted on mate selection in the female choice (a) and male choice (b) trials. Analysis was conducted using GLMs with quasibinomial distributions.

<b>TRIAL</b>	<b>FACTORS</b>	<b>LR<math>\chi^2</math></b>	<b>df</b>	<b>p</b>
<b>a) Female choice</b>	Male combination	0.1871	1	0.6653
	Female treatment	0.0006	1	0.9803
	Male treatment: Female treatment	0.2707	1	0.6029
<b>b) Male choice</b>	Female combination	0.9560	1	0.3282
	Male treatment	1.7439	1	0.1866
	Female combination: Male treatment	0.0316	1	0.8589

**Table 3.4.** Analysis conducted on mating latency in the no choice (a), female choice (b), and male choice (c) trials. Analysis was conducted using anovas. In the case where a significance was found we followed with a Kruskal-Wallis Rank Sums test and Tukey post-hoc.

<b>TRIAL</b>	<b>FACTORS</b>	<b>df</b>	<b>Mean sq</b>	<b>F</b>	<b>p</b>
<b>a) No choice</b>	(Mated and non-mated)				
	Male treatment	1	11762	2.269	0.133
	Female treatment	1	413	0.080	0.778
	Male treatment: female treatment	1	777	0.150	0.699
	(Mated only)	1	10091	4.403	<b>0.0374</b>
	Male treatment	1	469	0.204	0.6518
	Female treatment	1	3948	1.723	0.1912
	Male treatment: female treatment				
<b>b) Female choice</b>	(Mated and non-mated)				
	Male combination	1	53	0.010	0.9192
	Female treatment	1	26131	5.077	<b>0.0251</b>
	Male combination: female treatment	1	2162	0.420	0.5175
	(Mated only)				
	Male combination	1	831.0	0.264	0.608
	Female treatment	1	1864.1	0.592	0.443
	Male combination: female treatment	1	943.8	0.300	0.585
<b>c) Male choice</b>	(Mated and non-mated)				
	Female combination	1	42664	8.365	<b>0.0042</b>
	Male treatment	1	17789	3.488	0.0630
	Female combination: male treatment	1	2397	0.470	0.4937
	(Mated only)				
	Female combination	1	229.6	0.114	0.736
	Male treatment	1	1493.9	0.741	0.391
	Female combination: male treatment	1	17.7	0.009	0.926

**Table 3.5.** Comparisons in latency made between the four NDH combinations across the three (no choice (NC), female choice (FC) and male choice (MC)) trials (a-d). Due to non-normal distribution, analysis was conducted using anovas and, in the case where a significance was found, followed with a Kruskal-Wallis Rank Sums test and Tukey post-hoc (results not shown here).

TRIAL	FACTORS	LR $\chi^2$	df	p	NC	MC	FC
<b>a) 1:3 male + 1:3 female</b>	Trial (mated + non-mated)	19.211	2	<b>6.736x10<sup>-5</sup></b>	<b>ab</b>	<b>b</b>	<b>a</b>
	Trial (mated only)	3.3630	2	0.1861	na	na	na
<b>b) 24:1 male + 24:1 female</b>	Trial (mated + non-mated)	1.9842	2	0.3708	na	na	na
	Trial (mated only)	3.1759	2	0.2043	na	na	na
<b>c) 1:3 male + 24:1 female</b>	Trial (mated + non-mated)	2.9374	2	0.2302	na	na	na
	Trial (mated only)	8.9906	2	<b>0.0112</b>	<b>b</b>	<b>a</b>	<b>a</b>
<b>d) 24:1 male + 1:3 female</b>	Trial (mated + non-mated)	4.7005	2	0.0953	na	na	na
	Trial (mated only)	9.1112	2	<b>0.0105</b>	<b>b</b>	<b>a</b>	<b>a</b>

**Table 3.6.** Analysis conducted on mating duration in the no choice (a), female choice (b), male choice (c) trials. Analysis was conducted using anovas. In the case where a significance was found we followed with a Kruskal-Wallis Rank Sums test and Tukey post-hoc.

TRIAL	FACTORS	df	Mean sq	F	p
<b>a) No choice</b>	Male	1	364	0.068	0.7943
	Female	1	7243	1.355	0.2454
	Male: female	1	22025	4.122	<b>0.0434</b>
<b>b) Female choice</b>	Male combination	1	13477	2.625	0.1065
	Female treatment	1	36313	7.074	<b>0.0083</b>
	Male combination: female treatment	1	12	0.002	0.9613
<b>c) Male choice</b>	Female combination	1	533	0.093	0.760
	Male treatment	1	1353	0.237	0.627
	Female combination: male treatment	1	14729	2.584	0.109

**Table 3.7.** Comparisons in mating duration between the four NDH combinations across the three (no choice (NC), female choice (FC) and male choice (MC)) trials (a-d). Due to non-normal distribution, analysis was conducted using anovas and, in the case where a significance was found, followed with a Kruskal-Wallis Rank Sums test and Tukey post-hoc (results not shown here).

<b>TRIAL</b>	<b>LR<math>\chi^2</math></b>	<b>df</b>	<b>p</b>	<b>NC</b>	<b>MC</b>	<b>FC</b>
<b>a) 1:3 male + 1:3 female</b>	4.8579	2	0.0881	na	na	na
<b>b) 24:1 male + 24:1 female</b>	3.1526	2	0.2067	na	na	na
<b>c) 1:3 male + 24:1 female</b>	9.2333	2	<b>0.0099</b>	<b>ab</b>	<b>b</b>	<b>a</b>
<b>d) 24:1 male + 1:3 female</b>	10.7790	2	<b>0.0046</b>	<b>b</b>	<b>a</b>	<b>a</b>

**Table 3.8.** Analysis conducted on egg-laying rates ~16 hours post-mating in the no choice (a), female choice (b), and male choice (c) trials. Analysis was conducted using GLMs with quasipoisson distributions.

<b>TRIAL</b>	<b>FACTORS</b>	<b>LR<math>\chi^2</math></b>	<b>df</b>	<b>p</b>
<b>a) No choice</b>	Treatment	21.1450	3	<b>9.824x10<sup>-5</sup></b>
<b>b) Female choice</b>	Female treatment	1.1139	1	0.2912
	Successful male treatment	0.2625	1	0.6084
	Female treatment: Successful male treatment	0.5833	1	0.4450
<b>c) Male choice</b>	Male treatment	0.0156	1	0.9006
	Successful female treatment	3.6852	1	0.0549
	Male treatment: Successful female treatment	0.1195	1	0.7296

**Table 3.9.** Comparisons in egg-laying rates ~16 hours post-mating between the four NDH combinations across the three (no choice (NC), female choice (FC) and male choice (MC)) trials (a-d). Analysis was conducted using GLMs with quasipoisson distributions.

<b>TRIAL</b>	<b>LR<math>\chi^2</math></b>	<b>df</b>	<b>p</b>	<b>NC</b>	<b>MC</b>	<b>FC</b>
<b>a) 1:3 male + 1:3 female</b>	24.13	2	<b>5.757x10<sup>-6</sup></b>	<b>a</b>	<b>b</b>	<b>a</b>
<b>b) 24:1 male + 24:1 female</b>	21.283	2	<b>2.39x10<sup>-5</sup></b>	<b>b</b>	<b>b</b>	<b>a</b>
<b>c) 1:3 male + 24:1 female</b>	16.14	2	<b>0.0003</b>	<b>a</b>	<b>b</b>	<b>a</b>
<b>d) 24:1 male + 1:3 female</b>	30.744	2	<b>2.109x10<sup>-7</sup></b>	<b>b</b>	<b>b</b>	<b>a</b>

**Table 3.10.** Analysis conducted on egg-laying rates ~26 hours post-mating in the no choice (a), female choice (b), and male choice (c) trials. Analysis was conducted using GLMs with quasipoisson distributions.

<b>TRIAL</b>	<b>FACTORS</b>	<b>LR<math>\chi^2</math></b>	<b>df</b>	<b>p</b>
<b>a) No choice</b>	Treatment	10.5650	3	<b>0.0143</b>
<b>b) Female choice</b>	Female treatment	0.1164	1	0.7330
	Successful male treatment	0.6834	1	0.4084
	Female treatment: Successful male treatment	1.4113	1	0.2348
<b>c) Male choice</b>	Male treatment	0.0369	1	0.8477
	Successful female treatment	1.8277	1	0.1764
	Male treatment: Successful female treatment	1.0910	1	0.2962

**Table 3.11.** Comparisons in egg-laying rates ~26 hours post-mating between the four NDH combinations across the three (no choice (NC), female choice (FC) and male choice (MC)) trials (a-d). Analysis was conducted using GLMs with quasipoisson distributions.

<b>TRIAL</b>	<b>LR<math>\chi^2</math></b>	<b>df</b>	<b>p</b>	<b>NC</b>	<b>MC</b>	<b>FC</b>
<b>a) 1:3 male + 1:3 female</b>	13.575	2	<b>0.0011</b>	<b>a</b>	<b>ab</b>	<b>b</b>
<b>b) 24:1 male + 24:1 female</b>	7.7458	2	<b>0.0208</b>	<b>ab</b>	<b>a</b>	<b>b</b>
<b>c) 1:3 male + 24:1 female</b>	6.1007	2	<b>0.0473</b>	<b>a</b>	<b>a</b>	<b>a</b>
<b>d) 24:1 male + 1:3 female</b>	4.4342	2	0.1089	na	na	na

**Table 3.12.** Analysis conducted on total egg production in the no choice (a), female choice (b), and male choice (c) trials. Analysis was conducted using GLMs with quasipoisson distributions.

<b>TRIAL</b>	<b>FACTORS</b>	<b>LR<math>\chi^2</math></b>	<b>df</b>	<b>p</b>
<b>a) No choice</b>	Treatment	32.613	3	<b>3.887x10<sup>-7</sup></b>
<b>b) Female choice</b>	Female treatment	0.0802	1	0.7770
	Successful male treatment	0.8769	1	0.3490
	Female treatment: Successful male treatment	1.5217	1	0.2174
<b>c) Male choice</b>	Male treatment	0.0805	1	0.7766
	Successful female treatment	5.4850	1	<b>0.0192</b>
	Male treatment: Successful female treatment	0.1400	1	0.7083

**Table 3.13.** Comparisons in total egg production between the four NDH combinations across the three (no choice (NC), female choice (FC) and male choice (MC)) trials (a-d). Analysis was conducted using GLMs with quasipoisson distributions.

<b>TRIAL</b>	<b>LR<math>\chi^2</math></b>	<b>df</b>	<b>p</b>	<b>NC</b>	<b>MC</b>	<b>FC</b>
<b>a) 1:3 male + 1:3 female</b>	15.566	2	<b>0.0004</b>	<b>a</b>	<b>b</b>	<b>a</b>
<b>b) 24:1 male + 24:1 female</b>	8.0933	2	<b>0.0179</b>	<b>b</b>	<b>a</b>	<b>a</b>
<b>c) 1:3 male + 24:1 female</b>	2.1325	2	0.3443	na	na	na
<b>d) 24:1 male + 1:3 female</b>	7.5	2	<b>0.0235</b>	<b>ab</b>	<b>b</b>	<b>a</b>

**Table 3.14.** Analysis conducted on offspring survivorship on the 24:1 P:C media in the no choice (a), female choice (b), and male choice (c) trials. Analysis was conducted using GLMs with quasibinomial distributions.

<b>TRIAL</b>	<b>FACTORS</b>	<b>LR<math>\chi^2</math></b>	<b>df</b>	<b>p</b>
<b>a) No choice</b>	Treatment	7.7287	3	0.0520
<b>b) Female choice</b>	Female treatment	0.0231	1	0.8791
	Successful male treatment	0.7656	1	0.3816
	Female treatment: Successful male treatment	0.6379	1	0.4245
<b>c) Male choice</b>	Male treatment	0.2876	1	0.5917
	Successful female treatment	0.6884	1	0.4067
	Male treatment: Successful female treatment	0.3572	1	0.5501

**Table 3.15.** Comparisons in offspring survivorship on the 24:1 P:C media between the four NDH combinations across the three (no choice, female choice and male choice) trials (a-d). Analysis was conducted using GLMs with quasibinomial distributions.

<b>TRIAL</b>	<b>LR<math>\chi^2</math></b>	<b>df</b>	<b>p</b>	<b>NC</b>	<b>MC</b>	<b>FC</b>
<b>a) 1:3 male + 1:3 female</b>	14.864	2	<b>0.0006</b>	<b>ab</b>	<b>b</b>	<b>a</b>
<b>b) 24:1 male + 24:1 female</b>	71.406	2	<b>3.122x10<sup>-16</sup></b>	<b>b</b>	<b>b</b>	<b>a</b>
<b>c) 1:3 male + 24:1 female</b>	15.828	2	<b>0.0004</b>	<b>ab</b>	<b>b</b>	<b>a</b>
<b>d) 24:1 male + 1:3 female</b>	41.732	2	<b>8.672x10<sup>-10</sup></b>	<b>b</b>	<b>b</b>	<b>a</b>

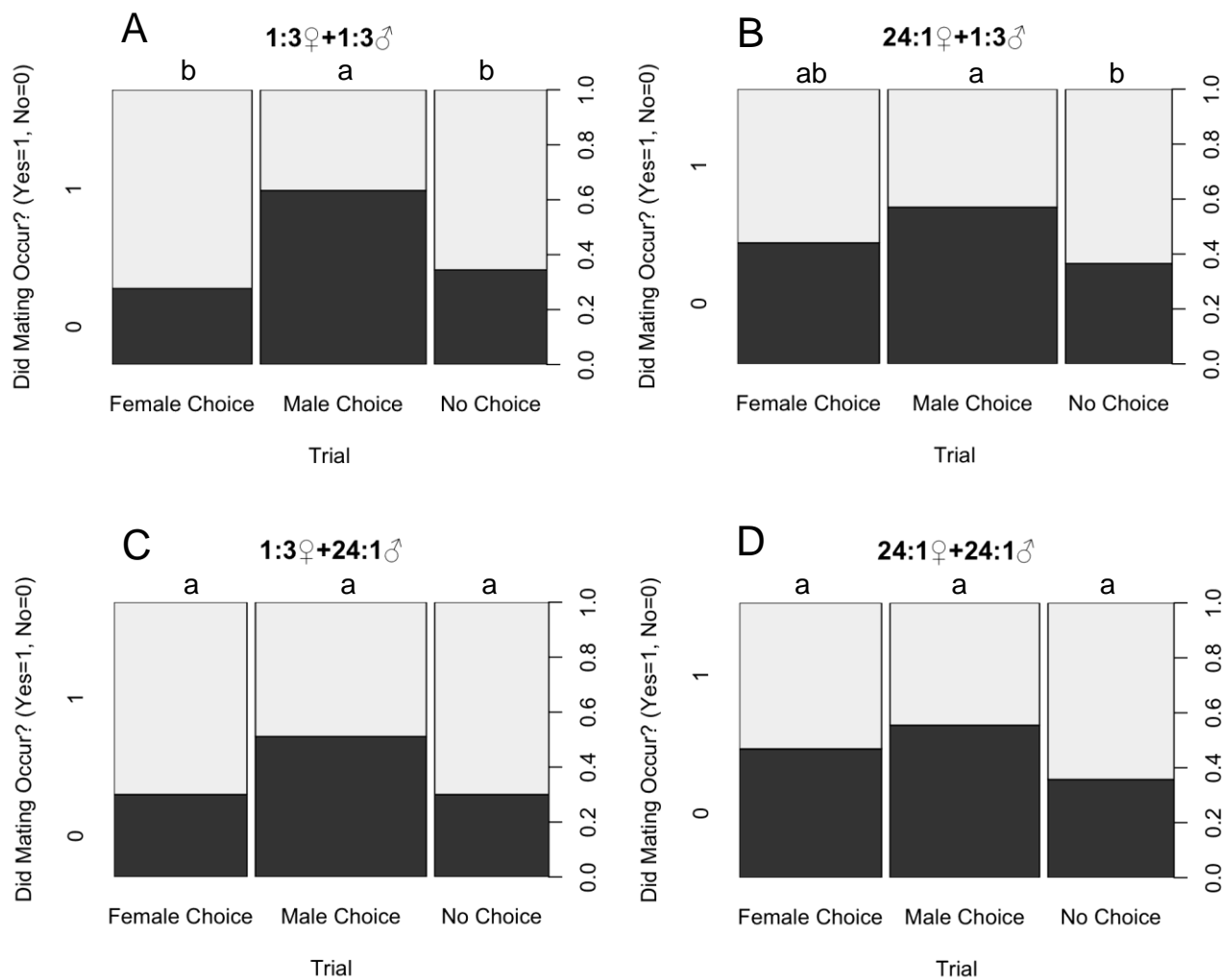
**Table 3.16.** Analysis conducted on offspring survivorship on the 1:3 P:C media in the no choice (a), female choice (b), and male choice (c) trials. Analysis was conducted using GLMs with quasibinomial distributions.

<b>TRIAL</b>	<b>FACTORS</b>	<b>LR<math>\chi^2</math></b>	<b>df</b>	<b>p</b>
<b>a) No choice</b>	Treatment	14.065	3	<b>0.0028</b>
<b>b) Female choice</b>	Female treatment	1.9247	1	0.1653
	Successful male treatment	0.9443	1	0.3312
	Female treatment: Successful male treatment	0.1173	1	0.7320
<b>c) Male choice</b>	Male treatment	0.0017	1	0.9676
	Successful female treatment	1.0323	1	0.3096
	Male treatment: Successful female treatment	0.0266	1	0.8705

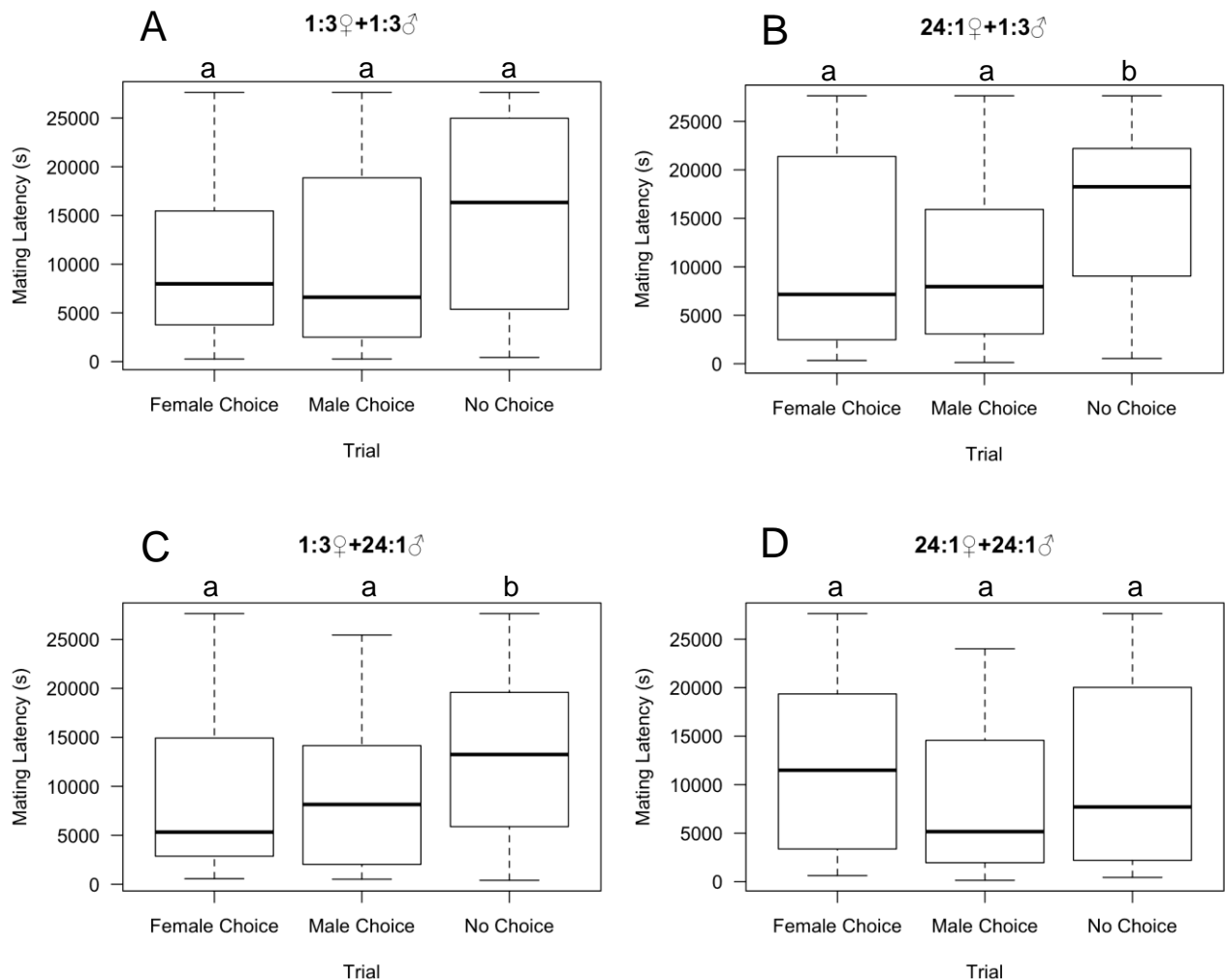
**Table 3.17.** Comparisons in offspring survivorship on the 1:3 P:C media between the four NDH combinations across the three (no choice, female choice and male choice) trials (a-d). Analysis was conducted using GLMs with quasibinomial distributions.

<b>TRIAL</b>	<b>LR<math>\chi^2</math></b>	<b>df</b>	<b>p</b>	<b>NC</b>	<b>MC</b>	<b>FC</b>
<b>a) 1:3 male + 1:3 female</b>	1.2468	2	0.5361	na	na	na
<b>b) 24:1 male + 24:1 female</b>	4.1674	2	0.1245	na	na	na
<b>c) 1:3 male + 24:1 female</b>	17.442	2	<b>0.0002</b>	<b>a</b>	<b>a</b>	<b>a</b>
<b>d) 24:1 male + 1:3 female</b>	1.5601	2	0.4584	na	na	na

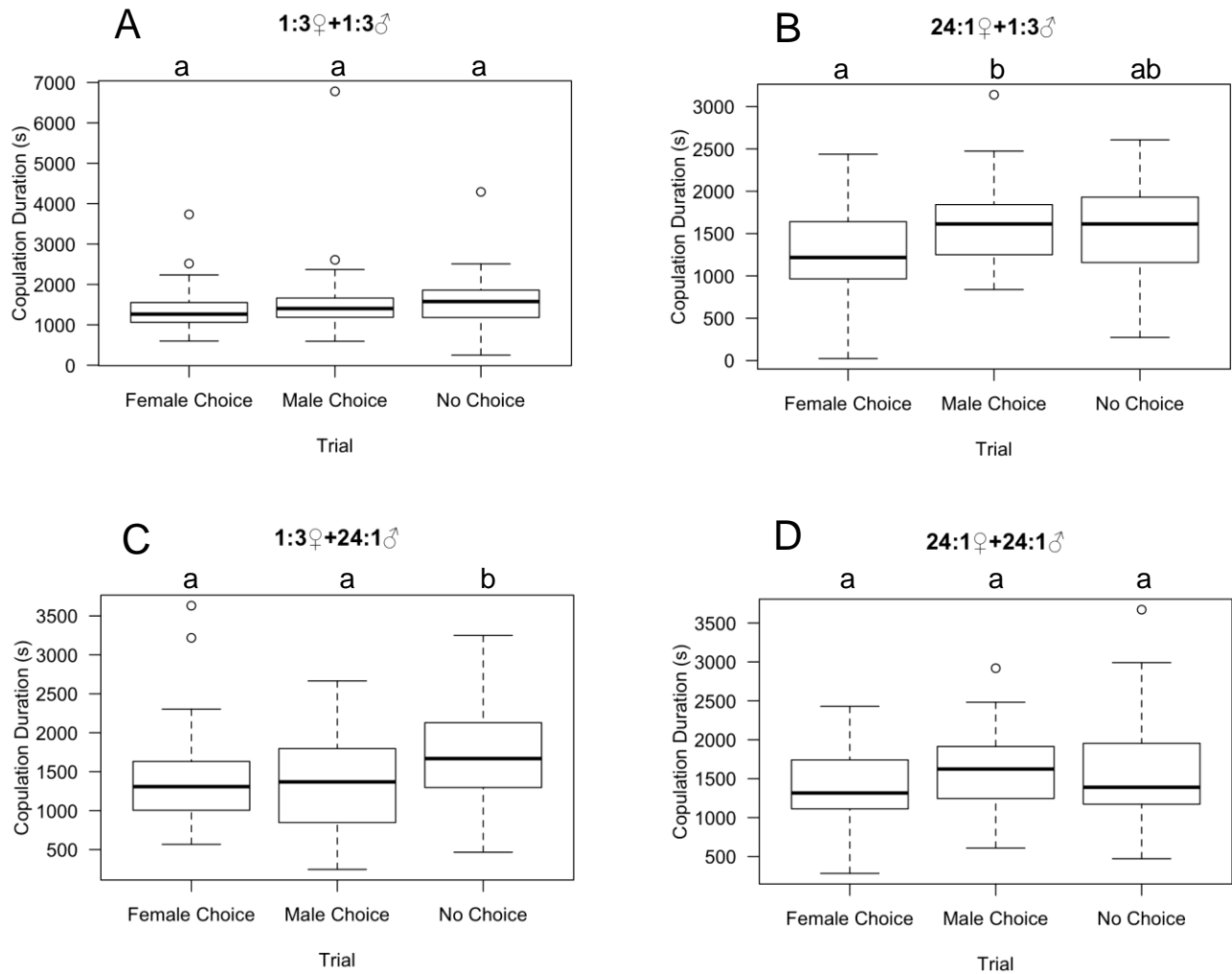




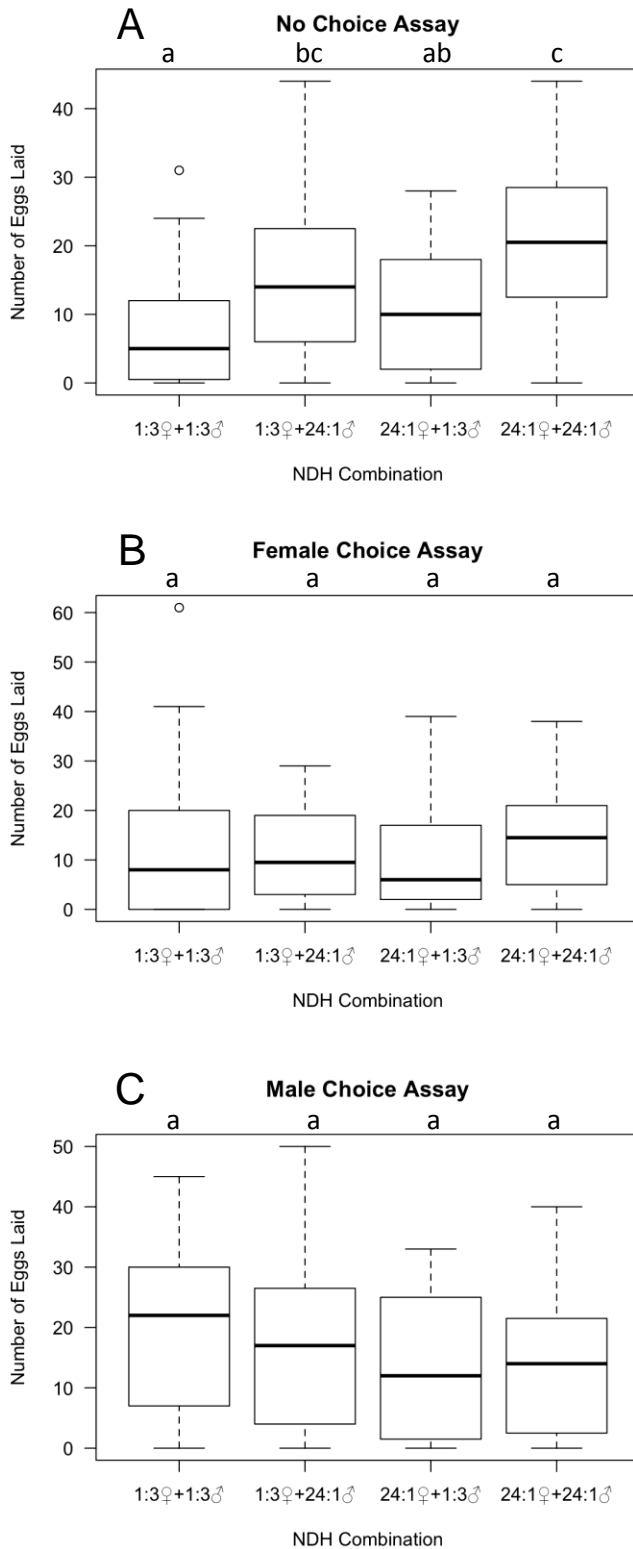
**Figure 3.1. Mating rates of *D. sukuzii* based on the nutritional developmental history (NDH) combination of flies in the three different assays.** Flies were raised on a low quality diet (1:3 flies) or a high quality diet (24:1 flies) to create two different phenotypes, creating four NDH combinations. The NDH combinations are 1:3 females with 1:3 males in (A), 24:1 females with 1:3 males in (B), 1:3 females with 24:1 males and in (C) and 24:1 females with 24:1 males in (D). Letters denote significant differences.



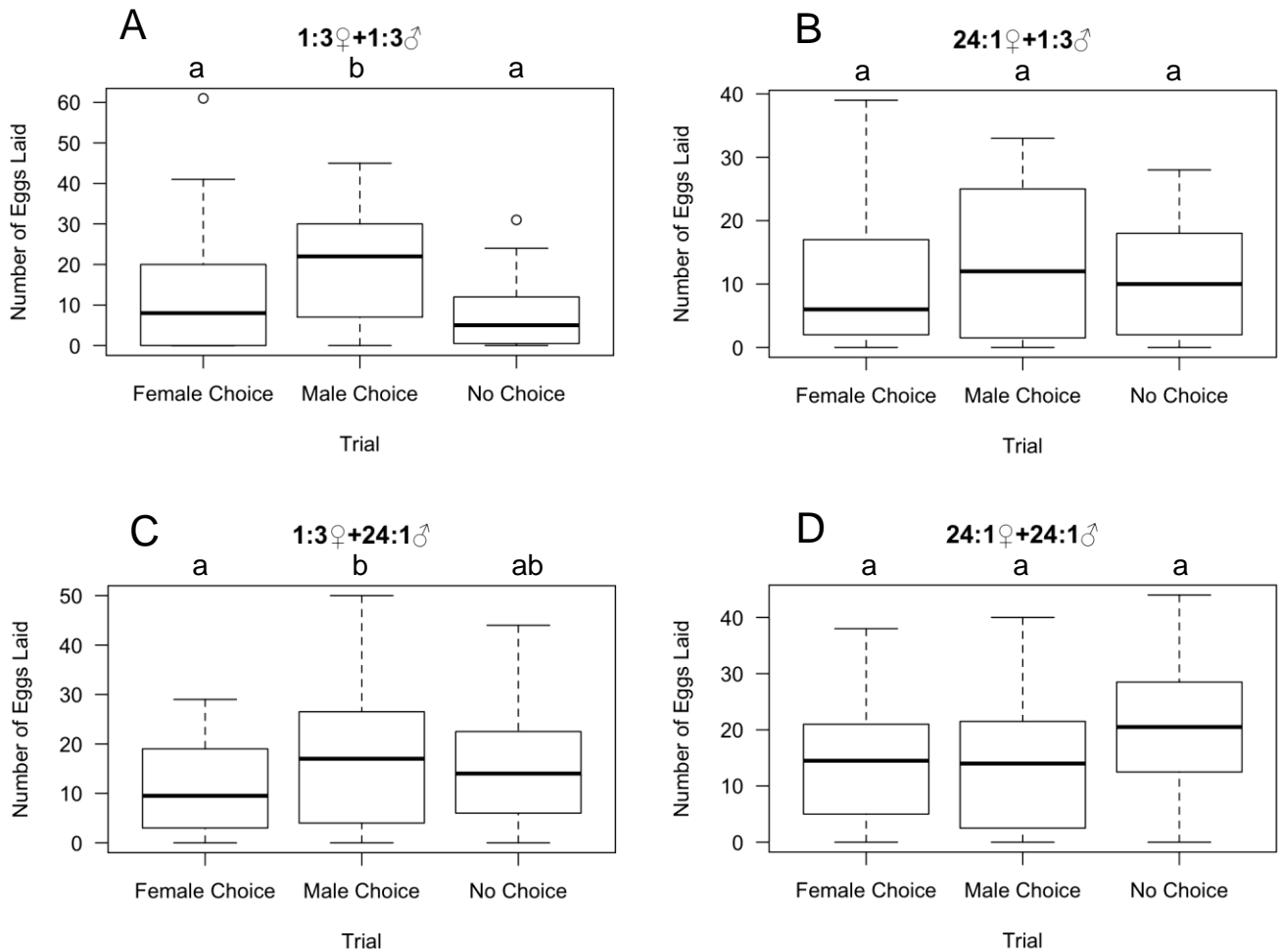
**Figure 3.2. Mating latencies (in seconds) of *D. sukuzii* based on the nutritional developmental history (NDH) combination of flies in the three different assays.** Boxplots of mating latencies from replicates where flies successfully mated. For all assays, flies were raised on a low quality diet (1:3 flies) or a high quality diet (24:1 flies) to create two different phenotypes, creating four NDH combinations. The NDH combinations are 1:3 females with 1:3 males in (A), 24:1 females with 1:3 males in (B), 1:3 females with 24:1 males and in (C) and 24:1 females with 24:1 males in (D). The box encloses values between the first and third quartiles of the data (the inter-quartile range (IQR)), whereas the horizontal bar within the box indicates the median. Whiskers extend from the box to largest/smallest values that are within  $1.5 \times$  the IQR of the box. Values outside that range are outliers and are indicated by circles. Boxplots that are not sharing a letter have significantly different medians.



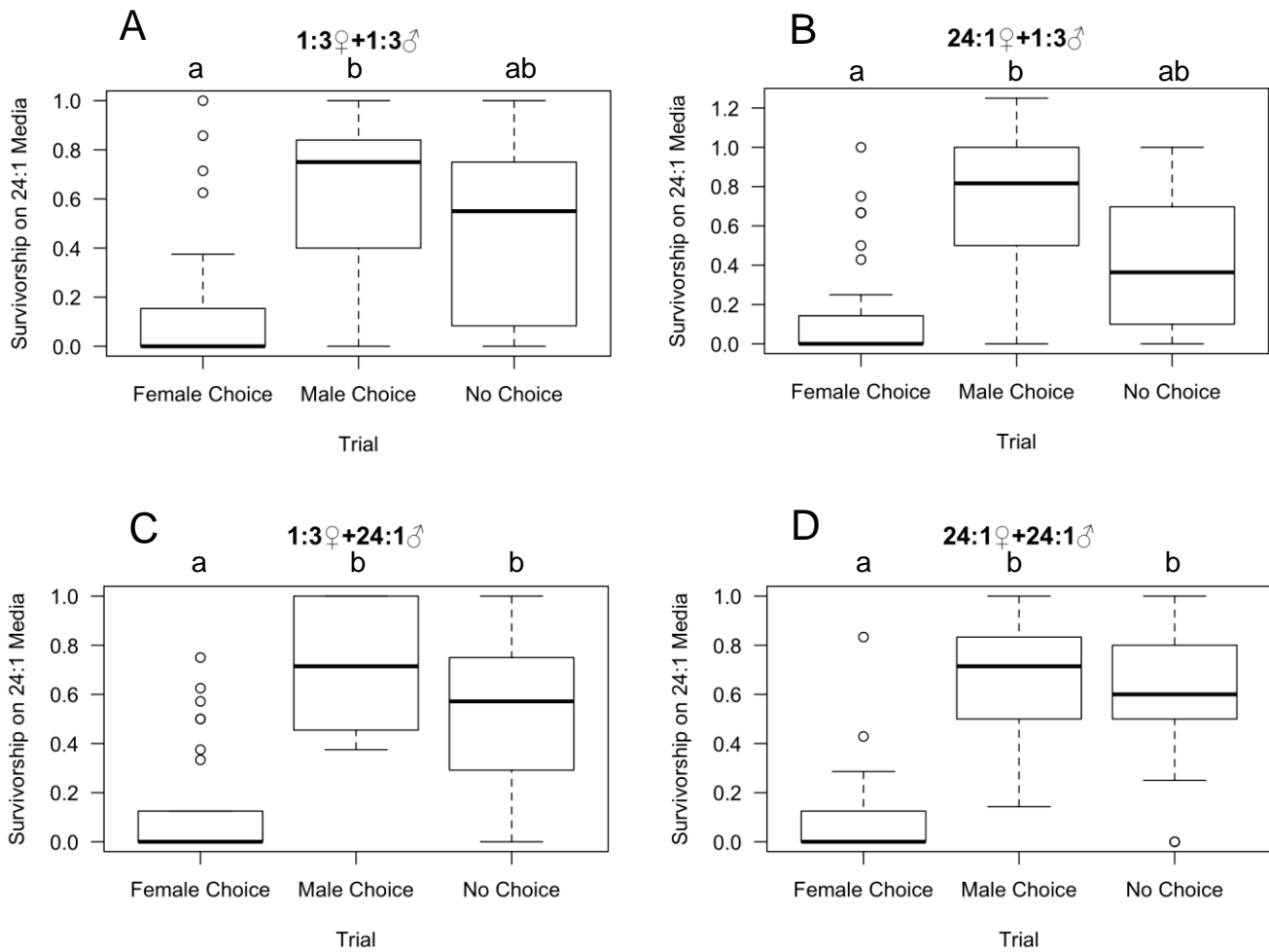
**Figure 3.3. Mating durations (in seconds) of *D. sukuzii* based on the nutritional developmental history (NDH) combination of flies in the three different assays.** Boxplots of the time spent during mating by flies of a particular NDH. In all assays, flies were raised on a low quality diet (1:3 flies) or a high quality diet (24:1 flies) to create two different phenotypes, creating four NDH combinations. The NDH combinations are 1:3 females with 1:3 males in (A), 24:1 females with 1:3 males in (B), 1:3 females with 24:1 males and in (C) and 24:1 females with 24:1 males in (D). The box encloses values between the first and third quartiles of the data (the inter-quartile range (IQR)), whereas the horizontal bar within the box indicates the median. Whiskers extend from the box to largest/smallest values that are within  $1.5 \times$  the IQR of the box. Values outside that range are outliers and are indicated by circles. Boxplots that are not sharing a letter have significantly different medians.



**Figure 3.4. Egg production in *D. sukukii* based on the nutritional developmental history (NDH) combination of flies in the three different assays.** Boxplots of the number of eggs laid by females over a 26 hour period following mating in the no-choice assay (A), female choice assay (B) and male choice assay (C). For all assays, flies were raised on a low quality diet (1:3 flies) or a high quality diet (24:1 flies) to create two different phenotypes, creating four NDH combinations. The NDH combinations are 1:3 females with 1:3 males, 24:1 females with 1:3 males, 1:3 females with 24:1 males and 24:1 females with 24:1 males. The box encloses values between the first and third quartiles of the data (the interquartile range (IQR)), whereas the horizontal bar within the box indicates the median. Whiskers extend from the box to largest/smallest values that are within  $1.5 \times$  the IQR of the box. Values outside that range are outliers and are indicated by circles. Boxplots that are not sharing a letter have significantly different means.



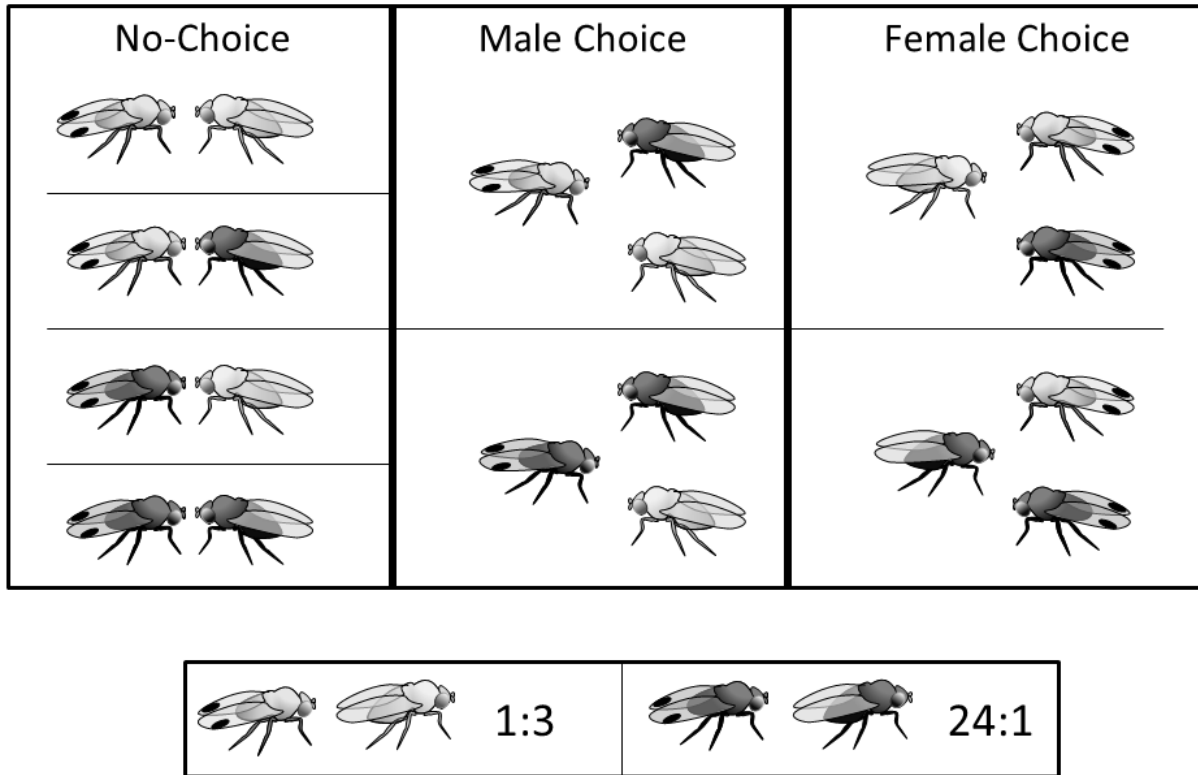
**Figure 3.5. Egg production in *D. sukuii* based on the nutritional developmental history (NDH) combination of flies in the three different assays.** Boxplots of the number of eggs laid by flies of a particular NDH over a 26 hour period following mating. In all assays, flies were raised on a low quality diet (1:3 flies) or a high quality diet (24:1 flies) to create two different phenotypes, creating four NDH combinations. The NDH combinations are 1:3 females with 1:3 males in (A), 24:1 females with 1:3 males in (B), 1:3 females with 24:1 males and in (C) and 24:1 females with 24:1 males in (D). The box encloses values between the first and third quartiles of the data (the inter-quartile range (IQR)), whereas the horizontal bar within the box indicates the median. Whiskers extend from the box to largest/smallest values that are within  $1.5 \times$  the IQR of the box. Values outside that range are outliers and are indicated by circles. Boxplots that are not sharing a letter have significantly different means



**Figure 3.6. Offspring survivorship on 24:1 media (high quality) in *D. sukuzii* based on the nutritional developmental history (NDH) combination of flies in the three different assays.**

Boxplots of the number of larvae that successfully developed into adults on the media consisting of a protein : carbohydrate ratio of 24:1. In all assays, flies were raised on a low quality diet (1:3 flies) or a high quality diet (24:1 flies) to create two different phenotypes, creating four NDH combinations. The NDH combinations are 1:3 females with 1:3 males in (A), 24:1 females with 1:3 males in (B), 1:3 females with 24:1 males and in (C) and 24:1 females with 24:1 males in (D). The box encloses values between the first and third quartiles of the data (the inter-quartile range (IQR)), whereas the horizontal bar within the box indicates the median. Whiskers extend from the box to largest/smallest values that are within  $1.5 \times$  the IQR of the box. Values outside that range are outliers and are indicated by circles. Boxplots that are not sharing a letter have significantly different means.

## Supplementary Figures



**Supplementary Figure 3.1** *The combinations of D. suzukii flies in the three different mating assays based on their nutritional developmental history (NDH).* Flies were either raised on a media with a protein to carbohydrate ratio of 1:3 (carbohydrate-rich; light-coloured flies) or 24:1 (protein-rich; dark-coloured flies) and organized into particular groups based on NDH and sex depending on the assay. Males have a single dark spot on each wing, whereas females do not.

## **CHAPTER 4**

### ON THE BEHAVIOURAL ECOLOGY OF AN INVASIVE SPECIES

In this thesis, I explored the potential importance of habitat and mate choice decisions on life history variation in *Drosophila suzukii* – a highly successful invasive species that is currently devastating soft fruit agriculture worldwide (Asplen *et al.* 2015). A parasite of fruit, *D. suzukii* inhabits environments that contain hosts (that often vary considerably in nutritional composition/quality) that are potentially available for oviposition, both spatially and temporally. Such differences have the potential to influence individual morphological, physiological and behavioural phenotypes important to fitness (Andersson 1994). Considering the (rather prodigious) success that *D. suzukii* have experienced, my thesis work is based on the premise that this species may owe some of its invasive ability to fitness-benefitting preferences in habitat(s) and mates. As such, the results of my experiments provide valuable insight into the biology of this species and in ways to improve current and future management techniques (e.g. through the manipulation of fly movement and population growth). In terms of assessing the relationship between habitat choice and fitness, I conducted a series of nutritional geometry experiments, in which female oviposition preference, fly association and larval performance was measured among different media that varied in their nutritional composition. Comparisons were then made between the results of these experiments to determine if *D. suzukii* provided offspring with the best developmental diet in accordance with the “preference-performance” hypothesis or “mother knows best” principle (Gripenberg *et al.* 2010). Utilizing knowledge about offspring performance from the previous assay, I then addressed *D. suzukii*'s ability to choose mates that provide the best individual fitness. In three separate experiments that differed in social context (no choice, female choice and male choice), I measured the mate preferences of flies that had



been raised on either a low or high quality diet, and were, presumably, of a low or high quality phenotype, respectively. Together, these assays uncover previously unknown details about this economically important species that can be of great use when planning future management strategies.

I found in the habitat choice and larval development experiments that adult *D. suzukii* prefer to associate and oviposit on media that is rich in carbohydrates, but that their offspring perform best when they are laid on media that that is rich in protein. Assuming that the nutritional composition of fruit remains the same following oviposition, then this observation suggests a possible conflict may be occurring between the life stages in this species. For example, rather than lay eggs that are best for offspring development, females may instead lay eggs wherever they obtain their own resources (*e.g.*, wherever there is ample sugar). Alternatively, in the event that the nutritional composition of the fruits following female oviposition does change (which will be of benefit to the larvae), then, rather than experiencing a conflict, *D. suzukii* have a “preference-performance” relationship between mother and offspring. Interestingly, in a second, follow-up developmental experiment, in which antimicrobials were not added to the media, larvae were found to perform almost equally well on the high-carbohydrate medias as they did on the high-protein medias. If this is the case, then targeting the growth of microbia in fruits following oviposition maybe a ‘fruitful’ trajectory in future control of this pest. In terms of current management, the fact that flies are highly attracted to sugary medias does suggest that trapping methods may be improved by the addition of sugary volatiles in baits. Additionally, observed differences in larval performance on the different ratios suggest that population growth is likely to be influenced by fruit variety and that crop design could help to reduce numbers.

In the mating experiments, I found that *D. suzukii* exhibited evidence of phenotypic plasticity related to both mating behavior and reproductive output. Flies appeared to discriminate between phenotypes as well as to adapt their behavior to improve personal reproductive success. This was supported by the observation that flies that developed on the lower quality diet (P:C of 1:3), and thus were presumably of a lower quality phenotype, were less attractive to flies that had developed on the higher quality diet (P:C of 24:1). Flies from the lower quality natal environment appeared to be less choosy about who they mated with, which suggests that *D. suzukii* may utilize a condition-dependent mating strategy to improve individual fitness, since being less choosy in this case may offset costs of potentially not getting a chance to mate (Cotton *et al.* 2006). The observation that lower quality flies are less choosy and less attractive also suggests that there is the potential for assortative mating to occur between *D. suzukii* flies of a lower quality phenotype. Although assortative mating has the potential to provide fitness advantages in the form of local adaptation (Bateson 1984) I did not see any clear evidence of this in regards to fecundity or larval survival. However, this may be due to flies of this phenotype having limited reproductive capacity. In terms of the importance of mate choice on individual fitness, I found conflicting results. On one hand, having a choice of mates was found to be beneficial in the case where females were mated to low quality males, yet choice also seemed to come at a cost to fitness, since females appeared to make poor mate choices. In other *Drosophila* species, sexual conflict plays a large role in mate choice (Parker 1979; Gowaty 1997; Gowaty & Buschhaus 1998; Holland & Rice 1998; Gavrilets *et al.* 2001) and the observation that females make poor mate choices suggests that *D. suzukii* may not be an exception to the “rule”. However, rather than females making poor choices, flies may instead be altering their reproductive investment (e.g., through the use of ‘bet-hedging’; see Garcia-Gonzalez *et al.* 2015) based on the

current social context and, thus, more research in this area is needed. In the field these observations may suggest that *D. suzukii* population growth is dependent on the interaction between the types of phenotypes present (low/high quality) and the social context (no-choice, female choice, male choice). Farmers, then, may benefit from exploiting this interaction to reduce population growth. For example, by promoting the immigration of low quality flies into populations of high quality flies.

***A multi-scenario situation: Habitat and mate choice combined***

When we integrate the results of both the habitat and mate choice assays, it is possible to envision several scenarios in which population growth rate is largely dependent on the nature of the female-offspring relationship, the size of the population, the types of hosts available and the occurrence of immigration/emigration. In the case where female oviposition preference *conflicts* with the dietary needs of juveniles (e.g., because the fruit does not become colonized by a protein-rich microbiotic community), then we might expect populations to consist of mostly low quality individuals when host fruits that are both high in sugar and low in protein are common. Size also matters; smaller sized populations are likely to exhibit the slowest growth rates due to reduced fecundity as a result of females mating with low quality males in a no-choice situation. However, the effect of size may be offset by the increased mating rates between low quality phenotypes due to lower choosiness and increased reproductive investment for competition with mates (Kvarnemo and Ahnesjo 1996). As the population grows larger we may then eventually see a slowing in the growth rate as flies reduce their reproductive investments (as a result of 'bet-hedging'). In the scenario where *D. suzukii* exhibits a *positive* relationship between female choice and offspring success (e.g., due to beneficial microbial colonization in the rotting fruit), then, alternatively, we might expect to see the opposite trend, where populations

consist of mostly high quality individuals when hosts that are high in sugar/low in protein are abundant. Contrary to the first scenario, in these populations fecundity might be higher in the no-choice situations compared to the choice situations, so we may expect to see the highest growth rate when populations are relatively small. As the population becomes larger, we might expect the growth rate to slow down due to greater choosiness in mates, and the effects of poor female choice/lower reproductive investment. As such, the scenario where the ‘preference-performance’ hypothesis does *not* apply, surprisingly, is potentially the one in which the fastest population growth occurs – that is, only when the population has reached a size where mate choice is an option. Considering the immense variations between landscapes inhabited by *D. suzukii*, however, and the temporal changes in host availability, another possible scenario is that the phenotypes that make up any given population become a mixture of low and high quality. This is because, regardless of the oviposition preference of females, populations of *D. suzukii* may potentially be ‘forced’ to oviposit on less preferred hosts at some time or another (due to a lack of alternative oviposition sites) and these low quality offspring then mix with members of the previous generation or from other populations via immigration/emigration. Thus, in these populations, we might expect to see a growth rate that is in-between that of the two scenarios described above. Overall, if there is one theme to unite all of these scenarios, it is that *D. suzukii* lives amongst a highly complex environment and that the plastic responses we see in development and mate choice suggest that a plastic response to environmental conditions may be an adaptive evolutionary strategy. As such, models predicting population growth rate and success in this species will need to take into account many different variables and their interactions with each other.

### *A final note and future directions*

In conclusion, the results of my experiments provide novel insights into the biology and behavior of *D. suzukii*. It has been made clear that flies have strong preferences for certain fruit-related compounds over others, which could, regardless of the nature of the mother-offspring relationship, have a significant influence on individual fitness. This may be through differences in offspring performance as well as through differences in adult phenotype, which ultimately, can determine population growth/success. In hindsight, however, because fruits vary greatly and certainly in much more than just their protein-to-carbohydrate ratio, a more complete understanding of this relationship requires that future research also investigate, in an integrated fashion, the behaviors of *D. suzukii* towards other fruit-related factors such as the concentration of protein and carbohydrates, colour, fruit-specific volatiles, penetration force, stage of ripeness etc. Similarly, other factors that could influence larval performance need to be investigated. Such variables include those that make up the host fruit chemical composition (e.g., micronutrients, plant-specific compounds etc.). As well, the prevalence and importance of microbial growth as a larval protein source will be an important avenue of research for management purposes, and which is likely to involve numerous different aspects including the nutritional quality of various microbial species and the success of colonization among different fruit varieties. In terms of sexual selection in *D. suzukii*, much more research is required to understand their reproductive behavior. The experiments I conducted were exploratory and involved only a select few of the many possible environmental and social variables to which flies in the wild will experience. Additionally, in order to better understand reproductive behaviors in this species, the possibility of poor female mate choice/variation in reproductive investment will need to be teased apart and other measures of fitness not measured in my experiments investigated (e.g., indirect benefits).

Finally, as there may be differences in behavior between populations based on differences in life history (as was observed!) over single and/or multiple generations, and/or due to differences in genetics, it will be important that the experiments reviewed here be repeated with flies that originate from other wild and lab-related origins for empirical support (and for fun!).

## References

- Asplen, M.K., Anfora, G., Biondi, A., Choi, D-S, Chu, D., Daane, K.M., Gibert, P., Gutierrez, A.P., Hoelmer, K.A., Hutchison, W.D, Isaacs, R., Jiang, Z-L, Karpati, Z., Kimura, M.T., Pascual, M., Philips, C.R., Plantamp, C., Ponti, L., Vetek, G., Vogt, H., Walton, V.M., Yu, Y., Zappala, L. and Desneux, Nicolas. 2015. Invasion biology of spotted wing *Drosophila* (*Drosophila suzukii*): a global perspective and future priorities. *Journal of Pest Science*, **88**: 469-494. doi 10.1007/s10340-015-0681-z
- Anderson, P., & Anton, S. 2014. Experience-based modulation of behavioural responses to plant volatiles and other sensory cues in insect herbivores. *Plant Cell Environ*, *37*: 1826-1835.
- Bateson, P. 1983. *Mate Choice*. Cambridge University Press.
- Cotton, S., Small, J., & Pomiankowski, A. 2006. Sexual selection and condition-dependent mate preferences. *Curr Biol*, *16*: 755-765.
- Garcia-Gonzalez, Francisco, Yukio Yasui, & Jonathan P. Evans. 2015. Mating Portfolios: Bet-Hedging, Sexual Selection and Female Multiple Mating. *Proc R Soc Lond Ser B Biol sci*, *282*: 20141525.
- Gavrilets, S., Arnqvist, G. & Friberg, U. 2001 The evolution of female mate choice by sexual conflict. *Proc R Soc Lond*, *B 268*, 531–539. doi:10.1098/rspb.2000.1382.
- Gowaty, P. A. 1997 Sexual dialectics, sexual selection, and variation in reproductive behavior. In *Feminism and evolutionary biology* (ed. P. A. Gowaty), 351–384. New York: Chapman & Hall.
- Gowaty, P. A. & Buschhaus, N. 1998 Ultimate causation of aggressive and forced copulation in birds: female resistance, the CODE hypothesis, and social monogamy. *Am Zool*, *38*: 207–225.
- Gripenberg., S., Mayhew., P. J., Parnell., M., and T. Roslin. 2010. A meta-analysis of preference-performance relationships in phytophagous insects. *Ecol Lett*. *13*: 383-393. doi:10.1111/j.1461-0248.2009.01433.x.
- Holland, B. & Rice, W.R., 1998. Perspective: chase-away sexual selection: antagonistic seduction versus resistance. *Evolution*, *52*:1-7.
- Kvarnemo, C., & Ahnesjo, I.1996. The dynamics of operational sex ratios and competition for mates. *Trends Ecol & Evol*, *11*: 404-408.

Parker, G. A. 1979. Sexual selection and sexual conflict. In *Sexual selection and reproductive competition in insects* (ed. M.S.Blum & N. A. Blum), 123–166. New York: Academic.