THESIS

THE ROLES OF PHENOTYPIC PLASTICITY AND ADAPTATION IN MORPHOLOGY AND PERFORMANCE OF AN INVASIVE SPECIES IN A NOVEL HABITAT

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ABSTRACT

THE ROLES OF PHENOTYPIC PLASTICITY AND ADAPTATION IN MORPHOLOGY AND PERFORMANCE OF AN INVASIVE SPECIES IN A NOVEL HABITAT

Invasive species spread and thrive across widely variable habitats. Their success in novel environments may be influenced by phenotypic plasticity, which occurs when a genotype can produce multiple phenotypes in response to different environments, or local adaptation, the production of traits that are advantageous under the local environmental conditions regardless of their effects in other habitats. One indication of these non-mutually exclusive processes comes in the form of geographic or elevational clines in phenotypes and genotypes. *Drosophilla suzukii* is an outstanding example of an invasive species that has established across many diverse environments and exhibits an elevational cline in wing size. In my thesis, with collaborators Jonathan Koch, Ian Pearse, Cameron Ghalambor, and Ruth Hufbauer, I evaluated the degree to which plasticity and genetic differentiation determine differences in wing sizes, and whether plasticity appears to be adaptive or not. I first characterized an elevational cline in wing size in *D. suzukii* on Hawaii and also evaluated its relative abundance by elevation. I then conducted a reciprocal temperature experiment to understand the mechanisms driving the cline. WIe found that wing size increased with elevation and that *D. suzukii* was significantly more abundant in higher elevation sites compared to lower elevation sites. Temperature may be the key driver of wing size variation, with wing size increasing as temperature decreased along the elevational gradient. In the reciprocal temperature experiment, I found that temperature had a strong effect on development time and cooler temperatures took longer to emerge compared to warmer temperatures. The reciprocal temperature experiment further revealed strong phenotypic plasticity. When flies from high and low elevation were reared at a cool temperature comparable to that found at high elevation, they produced larger wings. When reared at a warm temperature comparable to that found at low elevation, they produced smaller wings, which is the same pattern of variation observed in field populations. Additionally, I found significant differences in the number of flies that emerged from the two experimental temperatures. Flies from low and high elevation sites produced similar numbers of offspring at the cool temperature, while high elevation flies produced significantly more offspring at the warm temperature compared to the low elevation flies, despite that temperature being their home temperature. My study revealed strong plasticity in wing size, but no indication of local adaptation. If the wing phenotypes observed in high and low elevation populations in the field represent fit phenotypes, then this plasticity is adaptive. The flies may be exhibiting an "all-purpose genotype" where a fit phenotype is produced across the environmental conditions and there is no selection for adaptation to occur. As evidence continues to mount in support of the highly plastic responses of *D. suzukii* to temperature, particularly with respect to wing size, and the possible adaptiveness of this response, future studies need to make the direct connection between wing plasticity and adaptation. How an invasive organism responds to different environments determines the extent of its novel range and the places that it will impact. Hawaiian populations of D. suzukii exhibit substantial phenotypic variation in wing size, development time, and offspring production with some genetic component to that plasticity.

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DEDICATION

I dedicate this work to Mama and Ann for always supporting me in my big dreams, the village that raised me, and to the shoulders of brown giants I have stood on.

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CHAPTER 1

INTRODUCTION

Invasive species spread and thrive across widely variable habitats, even exceeding the range of environments that they experience in their native range (Broennimann et al. 2007). They may do this through a number of non-exclusive mechanisms including plastic shifts in response to their new environments and rapid adaptation (Richards et al. 2006, Felker-Quinn et al. 2012, Lande 2015). Phenotypic plasticity, when different phenotypes are produced by one genotype in response to the environment (Conner & Hartl 2004), itself can be adaptive, non-adaptive, or neutral in relation to an organism's fitness (Ghalambor et al. 2007). A plastic response to the environment is considered adaptive when the plastically produced phenotype increases fitness, non-adaptive when the phenotype decreases fitness, and neutral when the phenotype has no effect on fitness (Ghalambor et al. 2007). Although the presence of plastic traits does not imply its adaptiveness, plastic responses, like adaptation, often result in a better match between the organism and the environment. The degree to which phenotypic plasticity enables or inhibits adaptation to novel environments is of long-standing interest in evolutionary ecology (Ghalambor et al. 2007, Lande 2014, Perry et al., 2018).

Adaptive plasticity can facilitate successful establishment in novel habitats (Corl et al. 2018; Bock et al. 2018) and may lead to more fixed (non-plastic) phenotypes that are also adaptive (Geng et al. 2007; Scoville & Pfrender 2010; Bock et al. 2018; Corl et al. 2018). When an organism arrives in a new range, it faces novel selection pressures, which may vary across the range. Selection can result in traits that are advantageous in the local environmental conditions regardless of their effects in other habitats (Fry et al. 1996). This can result in higher relative fitness for an organism in its local environment in comparison to alternative ones, a process known as local adaptation (Kawecki & Ebert 2004). Although local adaptation improves fitness in local environments, it is not always advantageous, as it can impede use of other habitats (Ronce & Kirkpatrick 2001). The type of environment also affects whether or not local adaptation evolves. For example, if temporal variation in habitat quality is high, then the organism must constantly move for resources (Kisdi 2002), making phenotypic more favorable than local adaptation unfavorable. Alternatively, in a heterogeneous environment where there is reduced dispersal (Kisdi 2002), conditions for local adaptation are more favorable (Kawecki & Ebert 2004). Understanding the importance of phenotypic plasticity and local adaptation in this era of climate change is important because it can reveal whether plasticity allows populations to produce phenotypes that are near or away from traits found in a given environment (Ghalambor et al. 2007) and whether populations are adapted to their local environments.

Understanding underlying evolutionary mechanisms utilized by organisms during colonization events could help us understand why we observe specific phenotypic patterns such as clines. A cline is a gradient in phenotype or genotype over space (Huxley et al. 1983) and clines in organismal phenotypes are often found in response to latitudinal (Chown & Klok 2003) and elevational (Hodkinson 2005) gradients in the abiotic environments that populations experience. Clines are often considered to be evidence of adaptation to the gradient of conditions encountered. Temperature is an aspect of environment that is particularly important for many organisms to cope with and clines in response to temperature gradients provide a powerful way to evaluate species responses to changing conditions. In insects, elevational or latitudinal clines in wing and body size, in which insects produce increasing wing and body sizes across a gradient of decreasing temperature occur in multiple species (Hoffmann et al. 2003*)*.

The mechanisms driving such clines in insect size may be general to ectotherms (Atkinson, 1994; Klok & Harrison 2013). The ectotherm temperature-size rule is the inverse relationship between ectotherms and environmental temperatures (Klok & Harrison 2013). Increases in temperature typically speed development, which produces smaller cell sizes and thus smaller body (van der Have & de Jong 1996). For example, in *Drosophila melanogaster*, reductions in eyes, legs and wings in response to temperature have been explained by cell size reductions in the epidermis of each of the organs affected (Azevedo et al. 2002). Despite developmental constraints that might link growth rates and cell size, smaller body size at higher temperatures also may be beneficial, as smaller cell size allows for the reduction of oxygen need and diffusion distances (Atkinson et al. 2006). Other evidence of temperature-size relationships being adaptive rather than reflecting simple physiological constraint are that different populations often show different slopes in the relationship (Gilchrist et al. 2004; Klok & Harrison 2013), suggesting the relationship can be fine-tuned by selection, and some species do not exhibit the pattern at all (Walters & Hassall 2005; Arnett & Gotelli 1999). Despite possible benefits of shifts in ectotherm size with temperature, Zamudio et al. (1995) found that *D. melanogaster* males with large bodies (induced by low developmental temperature) were less successful than smaller flies at controlling resources. Thus, whether or not shifts in size with temperature are consistently beneficial remains an open question. Another species of *Drosophila* that may conform to the ectotherm temperature-size rule is *D. suzukii*.

Drosophilla suzukii is a striking example of an invasive species that has established across wide latitudinal and elevational gradients, in locations representing having abiotic environments different than experienced in the native range, suggesting an ecological niche shift in the introduced range (Fraimout et al. 2017; Orsted & Orsted 2019). It was introduced to Hawaii, USA from Asia in the 1980s (Kaneshiro, 1983) and has since spread across North America and Europe (Hauser, 2011; Calabria et al. 2012). Research on the abiotic niche and environmental tolerances of *D. suzukii* show that peak oviposition occurs around 25˚C, that larval development is most successful between 20-25˚C and finally that lifetime oviposition is highest at high humidity (70-95%) (Winkler et al. 2020). *Drosophila suzukii* on Hawaii have been found to produce larger wings at higher elevation and smaller wing sizes at lower elevations (Koch et al. 2019). Wing size in *Drosophila* species often corresponds to body size (e.g. Sokolof 1965; Azevedo et al. 1998; Gilchrist et al. 2004), and body size is often associated with reproductive potential, body fat content, and dispersal distance (Zamudio et al. 1995; Arrese & Soulages 2010; Roff 1977), thus changes in body size can help us understand its fitness at varying temperatures and elevation. Furthermore, larger wings of *D. suzukii* that developed in cool temperatures provide more lift and are considered advantageous for flight (Fraimout et al. 2018).

The known plasticity in wing size in *Drosophila* species, including *D. suzukii* (Fraimout et al. 2018), and the fast spread of this novel species nearly world-wide, makes this elevational cline in wing size is a great opportunity to evaluate the degree to which plasticity and genetic differentiation determine differences in wing sizes, and whether plasticity appears to be in the adaptive direction or not. We further explore what drives wing size variation in *D. suzukii* focusing on an elevational gradient on Hawaii island, USA, asking the following questions: How do size and abundance vary along elevational, seasonal and temperature gradients on Hawaii? To what degree are differences in size plastic responses due to temperature? Is there evidence that flies from low and high elevation are adapted to the temperatures they experience in the field? Is there evidence that phenotypic plasticity in wing size is adaptive?

To answer our questions, we collected *D. suzukii* along an elevational gradient on Hawaii Island, USA over the course of a season and measured wing size and tallied abundance. In addition, to better understand the underlying mechanism driving wing size, we collected flies from low and high elevation sites on Hawaii Island, USA, and used their descendants in a reciprocal transplant study, using temperatures representative of the collection sites. We evaluated whether *D. suzukii* size varied with temperature due to plastic responses to the environment (Fig. 1, H1), by collection elevation due to fixed genetic differentiation across environments (H2), plasticity in the presumably adaptive direction with genetic differentiation (H3), genotype by environment effects indicative of local adaptation to temperature (H4), or other patterns of variation.

METHODS

Wing size across elevation

Collections

Drosophila suzukii were collected from six sample sites along an elevational gradient in Hawaii County, USA in 2017 at five elevations (109 m, 668 m, 1147 m, 1523 m, 1772 m, 2,032 m) approximately monthly between February and November. Due to various constraints (for example, road construction) sites were not all sampled every month (Table S1). To sample, three traps were placed along a 50 m transect at each site for 7-14 days each. We used Haviland traps (Lee et al. 2013), which were made with a 32 oz food storage container, a screw top lid, plastic straws, a plastic plate, a thin rope and bait. A mixture of 7 grams of yeast, 14 grams of sugar and 250 milliliters of water were used as bait and placed inside the traps. A Thermochron (OnSolution Pty Ltd 2017) temperature sensor was taped on one trap per site to collect average temperature readings for sites at 109 m, 1523 m, 1,772 m, and 2,032 m for April, May and June 2017.

Wing phenotype measurements and fly abundance

Once the flies were collected, they were then identified and separated by sex (Walsh et al. 2011), and the right wing was then dissected and photographed under a dissecting microscope using a 1-millimeter stage micrometer. The photographs of the wings were mapped following the 15 points located at vein intersections found on Fig. 2 (Gidaszewski et al. 2009), and then measured using the 1-millimeter micrometer as a standard on Image J (Abramoff et al. 2012). The 15 points were then analyzed using Morpho J (Klingenberg 2011) to determine the centroid size (Dryden &

Mardia 1998) (henceforth referred to as "wing size"). In addition to measuring wing size, the flies collected from the traps were also counted to evaluate abundance by elevation in samples collected January through July (Table S1).

Reciprocal temperature experiment

Collections

Live *D. suzukii* were collected as eggs in fruits from low-altitude sites (<700 m; Fig 3) and high-altitude sites (> 1700 m; Fig. 3) along State Route 200 (also known as "Saddle Road") on Hawaii county, Hawaii USA in June 2019. Based on analyses of wing sizes across the elevational gradient (Fig 5), these elevations produced flies at the extremes of the phenotypes (Fig 3). At each site we set out three traps baited with fruit for the local population to oviposit into. Traps were made of a clear plastic cup container with a snap on lid, with four 3 mm holes around the side of the container that were 1.5 inches above the bait (Lee et al. 2012, Fig 4) and hung 1-2 m above the ground. Each of the three traps had a different bait (blueberries, raspberries or bananas) in an effort to capture the local population, which might exhibit differences in oviposition preferences. The traps were hung approximately 100 m apart and left in the collection sites for 24-72 hours. The fruit baits were then placed in an incubator that was set to 18°C, the average temperature between high elevation (14°C) and low elevation (22°C). Traps were put out at 20 low and 20 high elevation sites three times, and we successfully collected flies from 6 low elevation sites and 12 high elevation sites (Table 1). As flies emerged from the fruit bait, *Drosophila suzukii* individuals were separated from other species, and were placed into vials with media formulated for *Drosophila* that was topped with a thin layer of strawberry puree to encourage oviposition (see supplementary materials) to oviposit into for 24 hours at 18°C.

These are the generation zero (G0) flies. After 24 hours, the flies were removed from the vials and transferred to new vials, which were then kept in the incubator at the intermediate temperature of 18°C. This temperature was used for the G1 larval environment because we suspected temperature to be a major determinant of fly wing and body size (Fraimout et al. 2018, Koch et al. 2019). Given the fact that larger individuals typically have higher fecundity, a standard environment would help minimize differences in maternal effects in the reciprocal transplant experiment described below. The vials with media used for oviposition were then transported to Colorado State University in Fort Collins, Colorado USA to conduct the reciprocal temperature experiment.

Experimental procedures

Once the first generation (G1) flies started emerging, it was evident that some low elevation sites and one high elevation site had fewer than 5 offspring, which were sometimes all the same sex. To maintain data points for low elevation sites, flies from locations that were not more than 200m away from each other were combined. Combining the sites resulted in 4 low elevation sites and 11 high elevation sites for analysis. As the first-generation flies matured, they were verified to be *Drosophila suzukii*, and used in the reciprocal temperature experiment. Using first generation flies (G1) instead of flies directly caught in the field allowed us to minimize nongenetic maternal effects. Vial with 10 flies (5 males and 5 females) were prepared and split into two temperature treatments: 14°C, modeling the high elevation environment or 22°C, modeling the low elevation environment in a fully-reciprocal manner so that flies from both elevations experienced both environments (Fig. 3). The G1 flies were allowed to oviposit for 24 hours and then were removed and preserved. The vials that had been oviposited into were returned to their assigned temperatures. Three temporal blocks of the experiment were initiated over a 3-week period as G1 flies emerged. As the second generation of flies (G2) emerged they were removed from the vials as soon as they were mature enough to determine their sex, the date of emergence was noted, and then the flies were preserved in 75% ethanol for subsequent measurement of wing morphology as described above using a Nikon SMZ18 stereomicroscope with the Nikon DS-Fi2 camera microscope attachment (Nikon Metrology Inc, America). Emergence dates were used to calculate development time (days to emergence), and the number of flies that emerged (here after *emergence*) was used to evaluate overall performance.

Statistical Analyses

Data analysis and visualization were conducted with the R statistical program language (R Core Development Team 2018) with the assistance of the RStudio platform (RStudio Team 2016). Linear models were analyzed using the CAR package (Fox & Weisberg 2019). Mixed models were created using the lme4 package (Bates et al. 2015) and the lmerTest (Kuznetsova et al. 2017). The emmeans package (Lenth 2019) was also used to determine the differences in means in response to the experimental temperatures and to visualize the interactions for all the models. Model selection was done using AIC values from the base R function extractAIC (R Core Team 2018). Other data visualization figures in this publication were created using ggplot2 (Wickham 2016).

Wing size across elevations and average temperature

A total of 1,212 individual fly wings were measured (n_{Female}= 644, n_{Male}= 568). A linear model was fit to understand how wing size varied in response to elevation, sex and the interaction between elevation and sex. All three predictors were included as categorical variables. Although elevation could be considered a continuous variable, a comparison of models with it as continuous or categorical showed little difference in either AIC value or R^2. Furthermore, visual inspection of the data suggested that the relationship between wing size (as well as abundance, described below) and elevation was not necessarily linear. Treating elevation as categorical allowed predicted responses to vary appropriately with elevation.

We expected that much of the effect of elevation was due to differences in temperature at the different elevations, so we also explored that statistically. Thus, a separate linear model was fit to directly evaluate how wing size varied in response to average temperature and sex and their interactions, using the data from the four sample sizes at which temperatures were recorded once/month for three months. Temperature thus also replaced month in this model. Temperature is not the only aspect of the environment that changes with elevation, however. Elevation encompasses within it changes in temperature, plus changes in other abiotic factors (e.g. wind speed, precipitation, humidity) and biotic factors (e.g. abundance of other species of *Drosophila* [data not shown] and community composition, including resources for larval development). To evaluate the general importance of those other factors, we extracted the residuals from the temperature model, and ran an analysis on the remaining residual variation. Specifically, the response was the residual variation from the temperature model, and the other factors in the model were elevation, month and their interaction.

Abundance across elevations

To evaluate how *D. suzukii* abundance varies by elevation, we ran two analyses. First, we analyzed the subset of the data for which we had samples at every site for every month (elevations 109 m, 1523 m, 1772 m, 2032 m) from February to July. With that complete subset, we were able to examine the interaction between elevation and month in how they affect abundance. Second, we compared abundance at all sites in July only, with elevation the only predictor variable. Abundance was log transformed to reduce heteroskedasticity in the residuals and back transformed for the graph. Additionally, we added 1 to all data, to include the few sites at which not *D. suzukii* were caught.

Reciprocal temperature experiment

Linear mixed models were run to analyze how development time and wing size varied by source elevation (high/low), sex and experimental temperature (14˚C and 22˚C). For each of the models, interactions between main effects were also evaluated. To control for unobserved heterogeneity, we included temporal block as random effects. A generalized linear mixed model was used to analyze the emergence data, employing a Poisson distribution and log link. Output was back transformed to construct the graphs.

RESULTS

Elevational study

Wing size

A total of 1212 individual fly wings were measured from the 2017 collections (n_{Female}= 644, n_{Male}= 568). We found significant differences in wing size with respect to elevation ($F_{5,\,1191}$ = 109, $p < 0.001$), sex ($F_{1,\,1191}$=473; $p<0.001$) , month ($F_{9,\,1191}$=8; $p<0.001$) and a near significant interaction between elevation and sex ($F_{5,\,1191}$=2; $p =0.09$). Fly wing size increased with elevation and female flies ($\bar{x}_{female}$= 3.41 mm ± 0.0115 SE; Fig. 5a) were significantly larger compared to male flies ($\bar{x}_{male}$= 3.06 mm ± 0.0122 SE). Fly wing size also varied across months and there was a modest interaction between elevation and sex due to slight differences in how wing size in the two sexes responded to elevation (Fig 5a).

The effect of elevation on wing size may be largely due to the different temperatures found at different elevations. Further analysis confirmed that wing size increased as temperature decreased ($F_{1,\,280}$=128; $p<0.001$, Fig. 5b*)*. After taking account of the effects of temperature, we found that month ($F_{2,}$ $p<0.05$) still had a significant effect on wing size.

Abundance

We found a significant difference in the number of *D. suzukii* collected by elevation ($F_{4,\,125}$=56; $p < 0.001$*)*, month ($F_{5,\,125}$=8; $p<0.001$) and an interaction between elevation and month ($F_{1,\,125}$=5; $p<0.001$, Figure 5c*)*. Looking specifically at July where we had complete abundance data for all elevation sites, we found a significant effect of elevation ($F_{6,22}=194; p<0.001$, Supplemental

Figure S1*)*, which indicated higher abundance at high elevation sites and lower abundance at low elevation sites.

Reciprocal temperature experiment:

Development Time

There was a significant difference in development time in response to experimental temperature ($F_{1,117}$=820*;* p*<0.001)*. Flies emerged faster at warm (low elevation) temperatures (22℃) than in cool (higher elevation) temperatures (14℃) (Fig.6). Elevation of origin, sex and interactions between them, including with temperature, did not significantly affect development time.

Wing size

Wing size responded strongly to experimental temperature ($F_{1,1253}$=1144; $p < 0.001$*)* and varied by sex ($F_{1,1253}$=653; $p<0.001$) (Fig 7a). Consistent with our field data (Fig 5a), there was a significant interaction between experimental temperature and sex ($F_{1,1253}$=5; $p<0.05$) (Fig 7a), which indicated that female flies were much larger than male flies in both temperature treatment ($\bar{x}_{female\ (14°C)}$= 3.80 mm ± 0.0365 SE; $\bar{x}_{male(14°C)}$= 3.43 mm ± 0.0364 SE; $\bar{x}_{female\ (22°C)}$= 3.32 mm ± 0.0368 SE; $\bar{x}_{male(22°C)}$= 3.00 mm ± 0.0372 SE) (Fig 7a). There were also a significant interaction between experimental temperatures and elevation (the elevation at which the flies' ancestors were collected) ($F_{1,1253}$=6, $p<0.05$) (Fig 7b), which showed that no matter which elevation the flies came from, they changed their wing size in response to the environmental temperature they were exposed. Both high and low elevation flies produced smaller wings when grown in 22℃ (low elevation temperatures) and produced larger wings when grown in 14℃ (high elevation temperatures) (Fig.76b).

Emergence

Emergence was influenced by experimental temperature ($\mathcal{X}_1^2$=6; $p<0.05$), elevation ($\mathcal{X}_1^2$=12; $p<0.0001$) and an interaction between experimental temperature and elevation ($\mathcal{X}_1^2$ =59; $p<0.0001$). Emergence of flies from low and high elevation was similar at the high elevation temperature (14 ℃) while emergence of flies that originated from high elevation was higher than emergence of flies that originated from low elevation when reared at low elevation temperature (22℃) (Fig. 7).

DISCUSSION

We studied an elevational cline in wing size in *Drosophila suzukii* and conducted a reciprocal temperature experiment to understand the mechanisms driving the cline. We found that wing size increased with elevation, which coincides with previous findings on variation in *D. suzukii* wing morphology on Hawaii (Koch et al. 2019). In addition, we found that *D. suzukii* was significantly more abundant in higher elevation sites compared to lower elevation sites. Average temperature may be the key driver of wing size variation, with wing size increasing as temperature decreased along the elevational gradient. To understand mechanisms causing this morphological variation, we then conducted a reciprocal temperature study using *D. suzukii* collected from high and low elevations. Temperature had an effect on the development time of the larvae, with the higher temperature causing faster emergence times. We found that both high and low elevation flies develop faster at 22°C and slower at 14°C, which fits well with other studies that have looked into how temperature affects development time for *Drosophila suzukii* (Tochen et al. 2014). Strong plastic responses to temperature was also found in wing size. We found that when high and low elevation flies were reared at a cool temperature comparable to that found at high elevation, they all produced larger wings. When reared at a warm temperature comparable to that found at low elevation, they produced smaller wings, which match the wing cline observed in field populations. Additionally, we found significant differences in the number of flies that emerged from the two experimental temperatures. Flies from low and high elevation sites produced similar numbers of offspring at the cool temperature, while high elevation flies produced significantly more flies at the warm temperature compared to the low elevation flies despite that temperature being their home temperature. Our study found strong plasticity in wing size, but no indication of genetic differentiation in wing size in flies from low and high elevation. Below, we present possible inferences explaining the patterns we observed.

The size of an organism can give an important indication of fecundity and fitness (Roff 1981; Zamudio et al. 1995). Our findings for *Drosophila suzukii* support the temperature size rule for ectotherms (Atkinson 1994) and research on *D. suzukii* wing size (Fraimout et al. 2018; Koch et al. 2019). In ectotherms, the temperature-size rule is a phenomena where there is a negative relationship between environmental and body size (Klok & Harrison 2013). Evidence suggests that the shifts in size are often adaptive (Atkins et al. 2006). As elevation increases, temperature decreases, and this temperature gradient likely drives differences in body size we observed.

Environmental conditions along this elevational gradient could also explain some of the variation in wing size that we found. In higher altitudes, there could be stronger selection for wings with larger surface areas where wing loading (the weight of the organism divided by the area of its wing) is much greater (Norry et al. 2001, Fraimout et al. 2018). There is also a decrease in atmospheric pressure at high altitudes. In birds, larger wings relative to bodies can be found at higher elevation (Altshuler and Dudley 2006), but given their smaller size, insects may not be affected in the same way. In addition, we also observed that fruits were less readily available in high elevation sites compared to low elevations sites, which could mean longer flight times in order to feed and reproduce. Norry et al. (2001) found a similar situation for *Drosophila buzzatii* in Argentina where feeding and breeding resources were less abundant in higher elevations, which suggested that highland flies needed to be better adapted to flight and required larger wing loading. However, there is evidence that *D. suzukii* may be able to develop in non-fruit resources such as mushroom and bird manure (Stockton et al. 2019), so it may be possible that they are using food sources other than fruits at high elevation on Hawaii.

The atmospheric environment may also have an effect on *Drosophila suzukii*. The island of Hawaii has an inversion layer 50-70% of the time, which is located roughly between 1524 m – 2133 m (WRCC). An inversion layer occurs when warm air is held above cooler air, which inverts the normal temperature gradient found along the elevation (NOAA National Weather). The sites 1775 m and 2032 m were approximately where Hawaii island's layer would occur and the average temperatures for those locations were low (average low and high temperatures ranging from approximately 5℃–20℃). For *D. suzukii,* optimal fecundity has been found at 18℃ and 22℃, and the lowest fecundity at 14℃ (in blueberries, Tochen et al. 2014). Our field abundance data, as well as the experimental emergence data are strikingly different: we found higher abundance at lower temperatures, and higher emergence at 14℃ than at 22˚C.

The site at 1523 m is located right in the inversion zone and has a slightly average temperature than 1772 m – 2032 m. There, we captured fewer *D. suzukii*, particularly early in the year (Fig 5c). The smaller number of flies collected at 109 m and 1523 m may be linked to the high humidity and annual precipitation found at lower elevations on the east side of the island of Hawaii. Relative humidity affects organisms through its beneficial effects on body water maintenance and detrimental effects on evaporative cooling (Sayeed & Benzer 1996). *Drosophila melanogaster* are known to avoid highly saturated habitats (Sayeed & Benzer 1996). However, evidence for the effect of humidity on *D. suzukii* is suggests it is mainly beneficial. Tochen et al. (2016) found that *D. suzukii* densities increased with relative humidity, and oviposition was also highest at high humidity. These results are in contrast to our finding of low abundance found in lower elevation sites. It may be that the physical effects of rain (hampering foraging and oviposition activities), pathogens, or competition with other species accounts for the low abundance of *D. suzukii* at low elevation.

In this study we found support for strong plasticity in *Drosophila suzukii* wing size in response to temperature. Although the effect size was small, there was still an indication of genetic differentiation in wing size in flies from low and high elevation based on the significant interaction between experimental temperatures and elevation, but there was no indication of genotype by environment interactions in the form of local adaptation. Some evidence indicating genetic differentiation is interesting because *D. suzukii* has not been around Hawaii island for too long and seems to have started to evolve in such a short time. The high and low elevation flies are essentially able to fully reproduce each other's phenotype in their source environment (Fig 6a). The environmental conditions may either too heterogenous, or had high temporal fluctuations in habitat quality, which promoted high dispersal rates and did not favor local adaptation (Kawecki & Ebert 2004). Additionally, there could have been high gene flow between high and low elevation sites, which further constrain the evolution of genetic differentiation, including local adaptation (Kawecki & Ebert 2004).

If the wing phenotypes observed in high and low elevation populations do indeed represent the best phenotypes that can be produced by these populations in their respective environments, then this plasticity is adaptive (Fig 1a). The flies may be exhibiting an “all-purpose genotype” where a fit phenotype is produced across the environmental conditions, which could mean that stabilizing selection could be inhibiting genetic differentiation (Ghalambor et al. 2007). While the presence of plasticity in body size does not directly imply its adaptive value, in certain cases where adaptive plasticity is so good (ex. *Pennisetum setaceum* introduction on Hawaii; Williams et al. 1995) it has been found to prevent adaptive evolution due to lack of directional selection, which leads to no evolution (Ghalambor et al. 2007), thus no genetic differentiation. To date, a direct connection between plasticity in wing size in *D. suzukii* with fitness is still to be determined, however there is evidence the larger wings are beneficial for flight. Fraimout et al. (2018) found that the larger wings *D. suzukii* produced when larvae develop at low temperature increase flight speed and acceleration, supporting the notion that plastic shifts in wing morphology have adaptive value. In addition, the relationship between fecundity and size seems to be most affected by phenotypic size as larger flies have been found to lay eggs sooner (Alpatov 1932), which changes fitness by altering fertility rates (Roff 1981). Our experimental design could not produce a direct link between wing size and fitness because answering that question would require two different evaluations of adaptive plasticity (or not) with two different phenotypes (wing size and number emerged), so we could not make direct inferences about the relative roles of adaptive plasticity and physiological constraint in determining wing size.

Emergence of flies from our reciprocal temperature experiment indicates reproductive performance in a standardized environment, integrating over oviposition and larval and pupal survival. *Drosophila suzukii* from high elevation were able to maintain relatively high and comparable emergence, whether they were in the cool or the warm temperature. In contrast, our experiment showed plasticity in *D. suzukii* from low elevation for the number of flies that emerged, but not necessarily in an adaptive direction, as they produced fewer flies in their home temperature of 22°C (Fig 7). This is a curious result, particularly given data from other populations of *D. suzukii* indicating that temperatures of 22˚C or more are better than lower temperatures for increasing oviposition and development time and success, as discussed above (Tochen et al. 2014; Winkler et al. 2020). The abundance data from field populations suggest a hypothesis to explain the low emergence of low elevation flies from warm temperatures. We found that *D. suzukii* was less abundant at low elevations, which may have resulted in more inbreeding and genetic drift and concomitantly higher genetic load in those populations. If this is the case, the low elevation population may have been unable to maintain homeostasis, resulting in low emergence rates.

For *Drosophila melanogaster,* two hypotheses have been presented to explain phenotypic responses to temperature: the 'acclimation-advantage hypothesis' (Leroi et al. 1993) and the 'size-advantage hypothesis' (Zamudio et al. 1995). The acclimation-advantage hypothesis proposes that organisms that are acclimated to an environment have an advantage over others that are not (Leroi et al. 1993). Additionally, acclimation effects may sometimes persist for several generations (Huey et al. 1995). Although the G1 flies were raised in 18°C after collection from the wild to standardize maternal environment, there could still be effects of acclimation to grandmaternal environment. In our reciprocal temperature study, despite low elevation field populations that were the source of our G0 flies being acclimated to warm temperatures, the low elevation population produced fewer flies than the high elevation population (Fig 7). Thus, our results do not support the acclimation-advantage hypothesis, though further tests with different acclimation temperatures just prior to performance tests would be needed to confirm lack of support. Our finding coincides with Zamudio et al. (1995), in which *Drosophila melanogaster* did not perform better in its acclimated temperature and concluded that acclimation does not guarantee improved performance. The size-advantage hypothesis posits that larger flies produced from cooler temperatures should be more successful due to their size (Zamudio et al. 1995). The high elevation flies in our experiment do exhibit support for the size-advantage hypothesis, in that they performed well in both cool and warm temperatures, perhaps due to their large size. This finding is in contrast to Zamudio et al. (1995), in which for *Drosophila melanogaster* bigger was not better with respect to guarding resources.

From our study, we have begun to understand the interplay between development time and wing phenotypes in *Drosophila suzukii*. As evidence continues to mount in support of the highly plastic responses of *D. suzukii* to temperature, particularly with respect to wing size, and the possible adaptiveness of this response, future studies should further evaluate the relationship between wing plasticity and fitness.

TABLES

Table 1. Collection locations sampled in 2019 along with the GPS coordinate, elevation, the type of elevation we considered it as for the reciprocal temperature experiment, and the number of male (M) and female (F) *Drosophila suzukii* collected per location.

Latitude	Longitude	Elevation (m)	Elevation	M	F
19.682	-155.187	674	low	12	3
19.683	-155.190	684	low	17	12
19.684	-155.192	690	low	2	1
19.685	-155.192	692	low	21	14
19.687	-155.194	698	low	14	4
19.689	-155.195	703	low	10	1
19.675	-155.384	1765	high	17	16
19.677	-155.384	1765	high	8	2
19.671	-155.384	1766	high	8	12
19.678	-155.385	1771	high	5	6
19.670	-155.386	1775	high	6	6
19.678	-155.386	1780	high	9	5
19.682	-155.396	1826	high	5	5
19.682	-155.398	1831	high	10	2
19.674	-155.466	2027	high	6	2
19.676	-155.465	2029	high	11	4
19.676	-155.465	2031	high	5	5
19.673	-155.467	2038	high	33	20

FIGURES

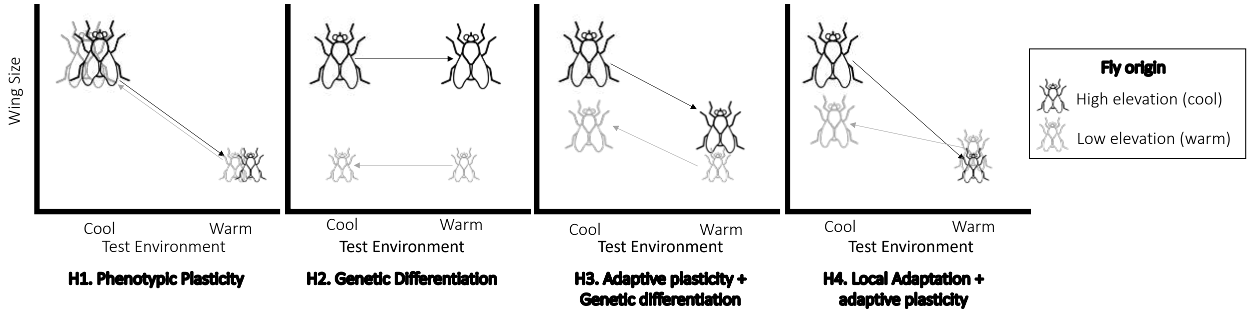

Fig 1 Experimental hypotheses for the observed variation in wing size for *Drosophila suzukii*. Wing size could vary with elevation due to plastic responses to the environment (H1), genetic differentiation due to adaptation to the environment (H2), or a combination of plasticity and population-level genetic differentiation where the plasticity could either be in the adaptive direction (H3) or organisms are best adapted to their local environment (H4).

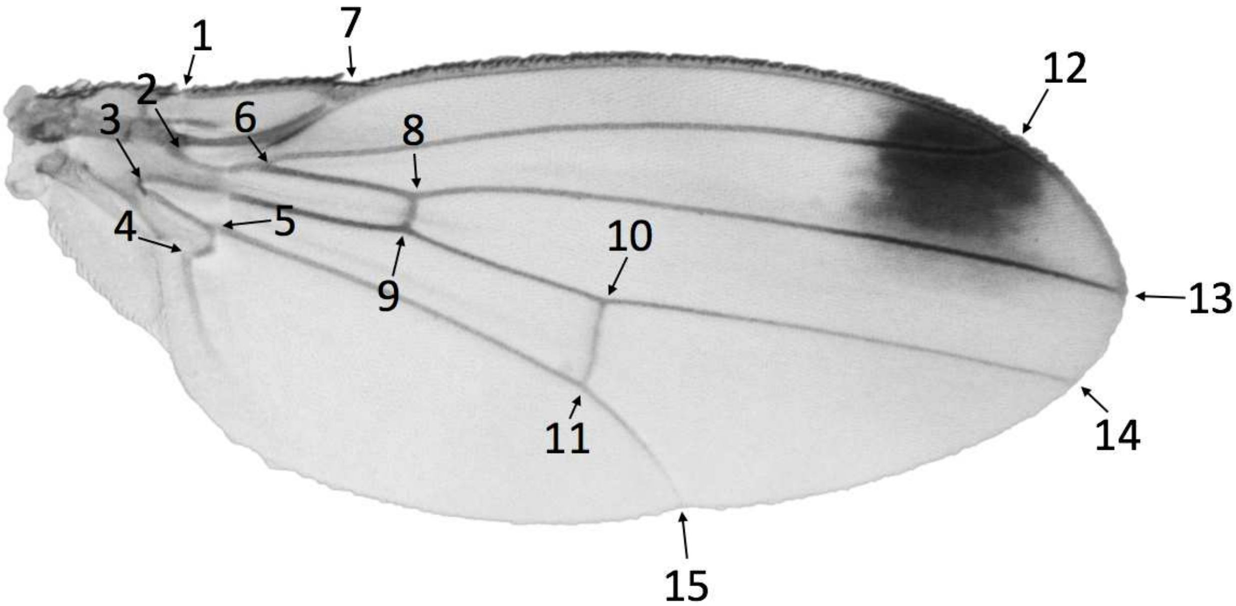

Fig 2 The fifteen landmarks used to measure wing size.

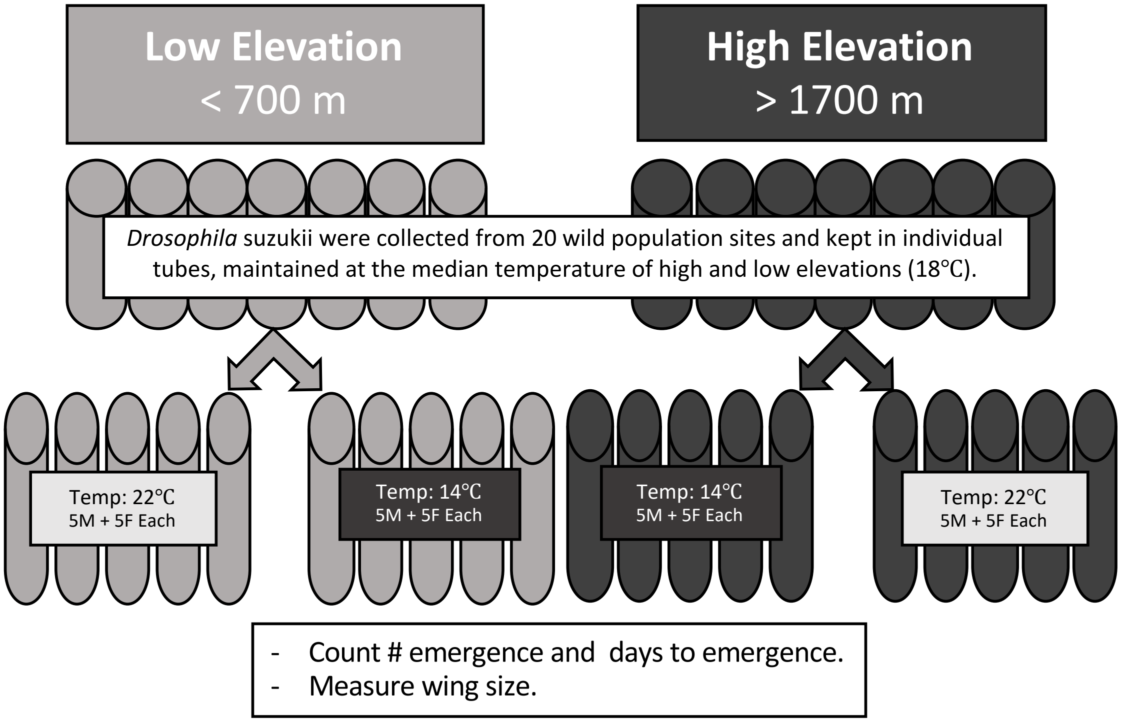

Fig 3 Experimental design. Flies from low elevation (<700m) and flies from high elevation (>1700m) were collected, placed in media, and placed in the growth chamber with the temperature set at 18℃ (G0). The flies that emerged were called G1 and placed in vials for the reciprocal temperature experiment. Each vial consisted of no more than 5 males and 5 females. The flies were then allowed to copulate and oviposit eggs in their assigned temperatures and removed after 24 hours. Their offspring (G2) that emerged were then counted and their wing size were measured.

Fig 4 Collection traps were made out of clear plastic containers with snap on lids with 3mm holes on the side of the container and 1.5 inch above the bait.

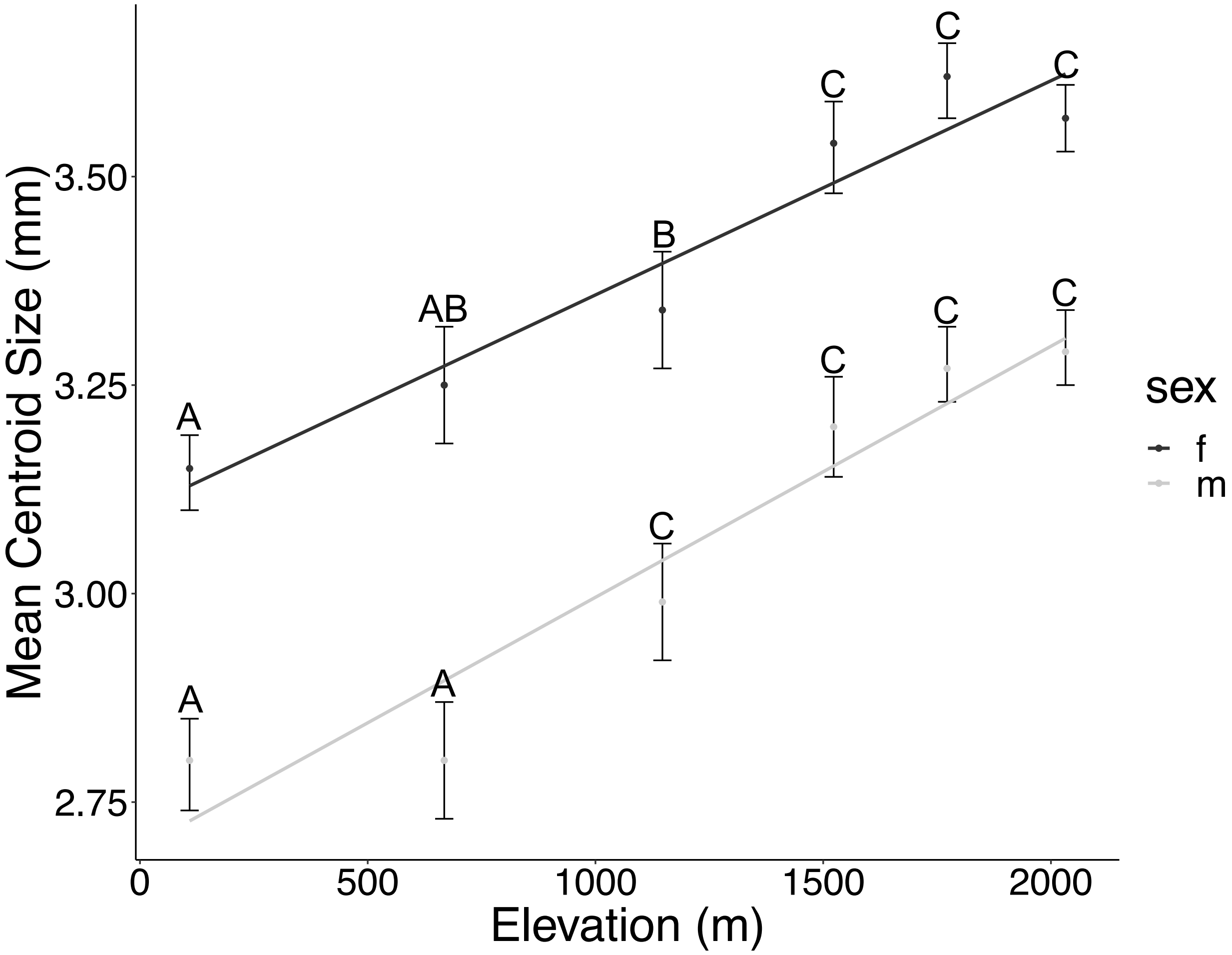

Fig 5a Relationship between elevation and wing centroid size of male and female *Drosophila suzukii*. We found significant differences in wing size with respect to elevation ($F_{5,1191} = 109$, $p < 0.001$), sex ($F_{1,1191}=473$; $p<0.001$) , month ($F_{9,1191}=8$; $p<0.001$) and a near significant interaction between elevation and sex ($F_{5,1191}=2$; $p =0.09$).

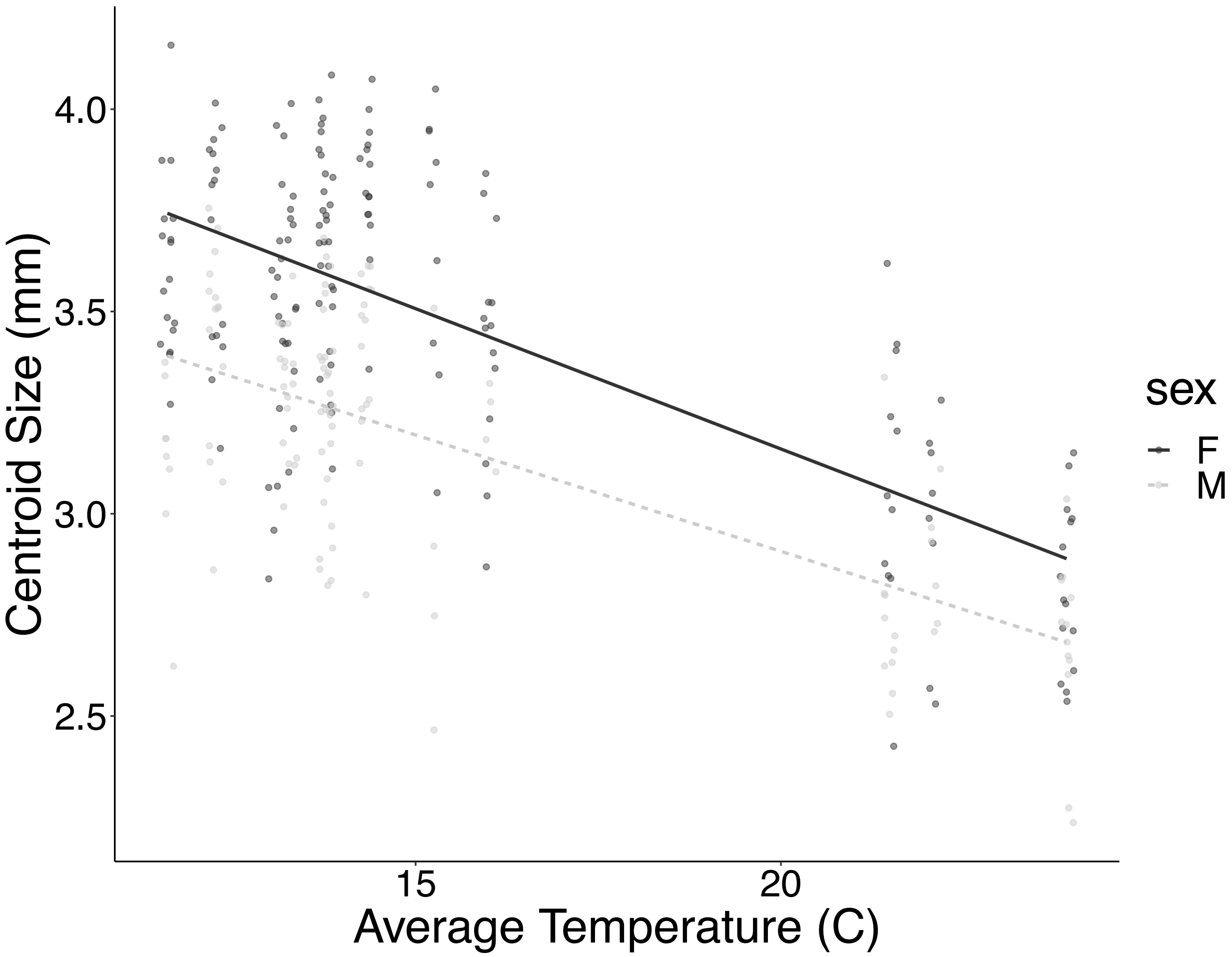

Fig 5b Relationship between the average temperature at the collection site and the wing centroid size of male and female *Drosophila suzukii*. We found that wing size increased as temperature decreased ($F_{1, 280}$=128; *p<0.001)*. The model means for each sex is indicated by the black and grey point on the graph.

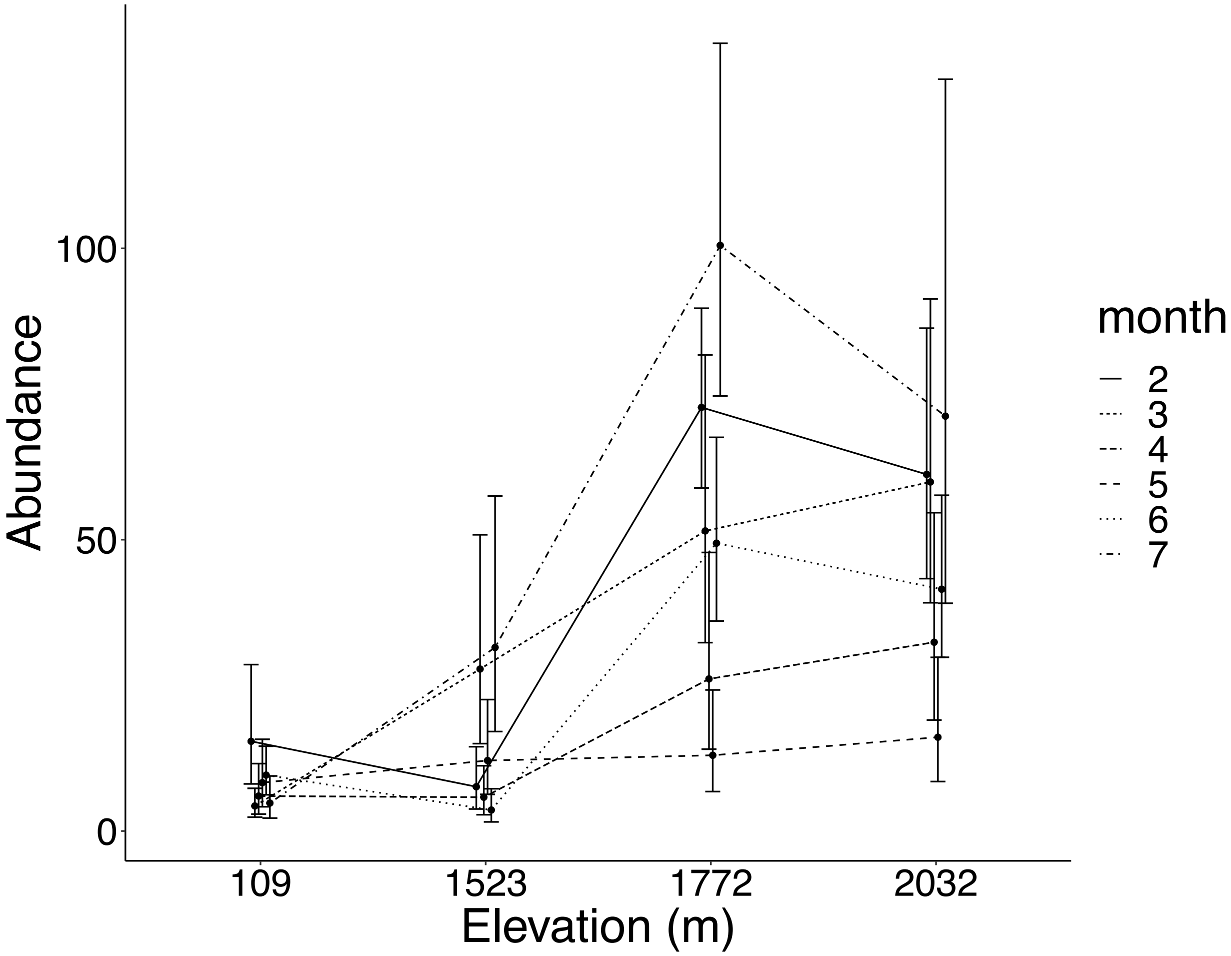

Fig 5c Relationship between elevation, month (February – July) and abundance of *D. suzukii* on Hawaii, showing back-transformed model means and 95% CIs, jittered by elevation to allow visualization of the points. We found a significant difference in the number of *D. suzukii* collected by elevation ($F_{4,125}$=56; *p <0.001)*, month ($F_{5,125}$=8; *p<0.001*) and an interaction between elevation and month ($F_{1,125}$=5; *p<0.001)*.

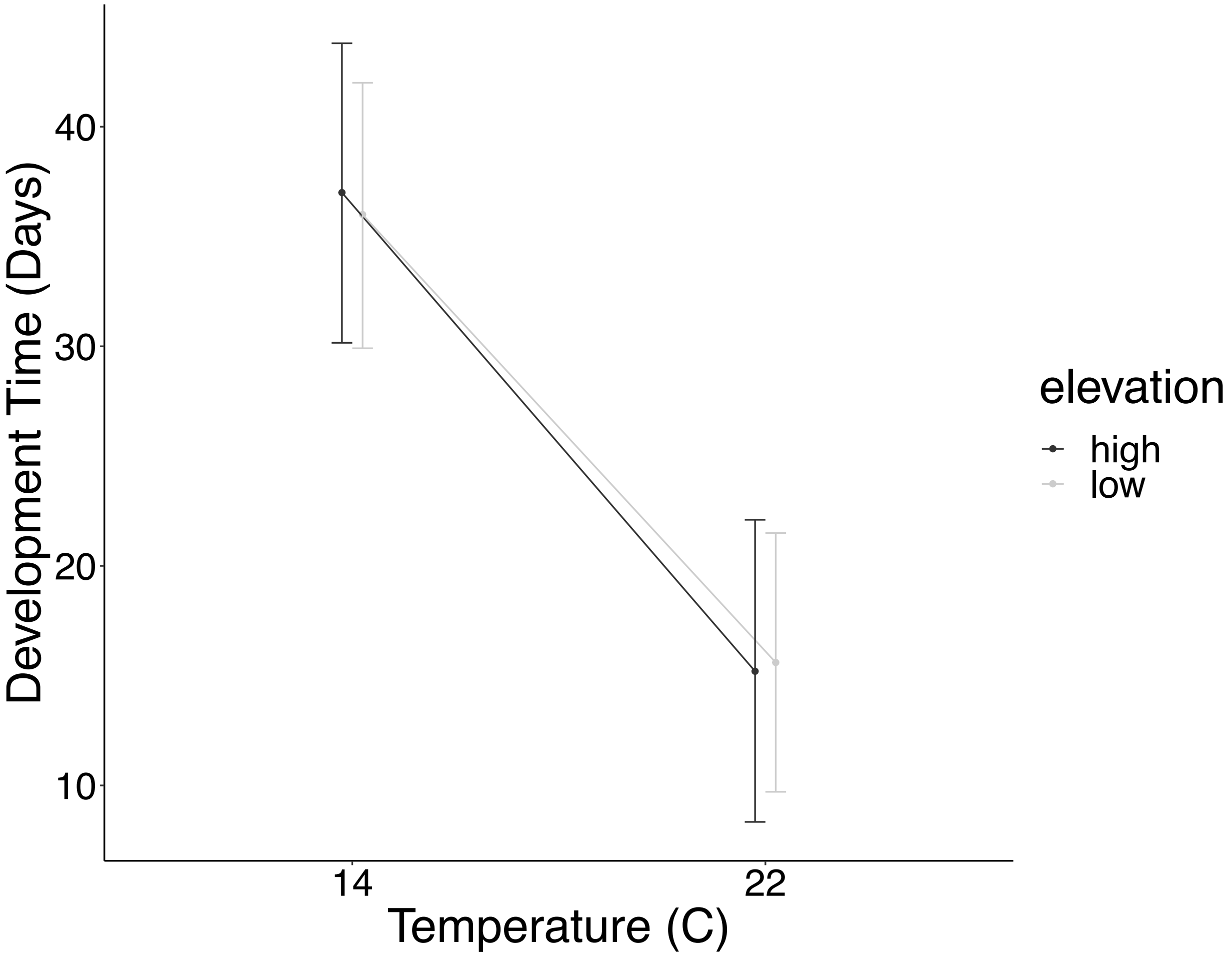

Fig 6 The effects of reciprocal temperature experiment on the development time (the number of days it took to emerge) on *Drosophila suzukii*. There was a significant difference in development time in response to experimental temperature ($F_{1,117}$=820; $p<0.001$)

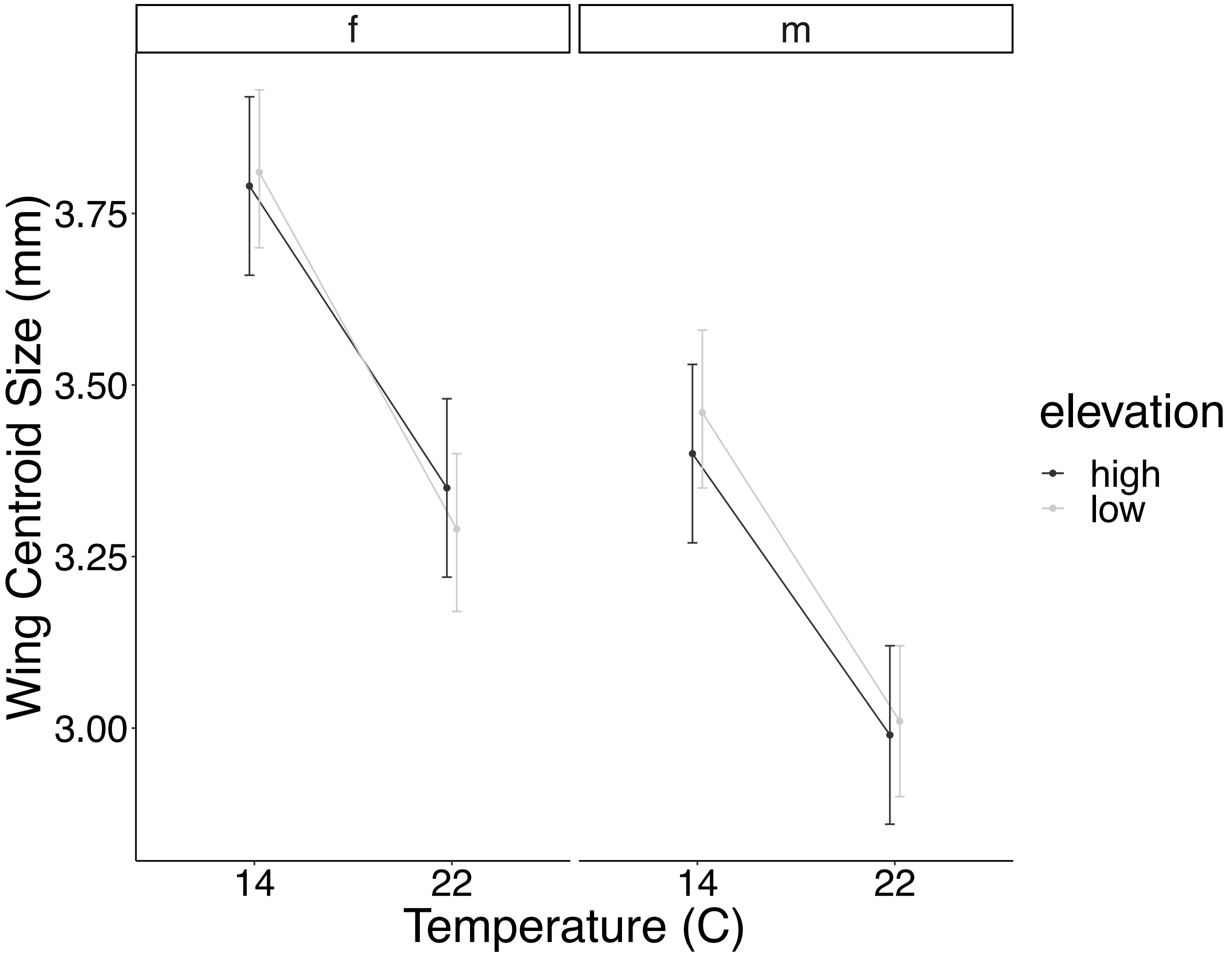

Fig 7a The effects of the reciprocal temperature experiment on the mean centroid size (wing size) of *Drosophila suzukii*. Wing size responded strongly to experimental temperature ($F_{1,1253}$=1144; $p<0.001$) and by sex ($F_{1,1253}$=653; *df*=1253; $p<0.001$). There were also significant interactions between experimental temperatures and elevation ($F_{1,1253}$=6; $p<0.05$), and between elevation and sex ($F_{1,1253}$=5; $p<0.05$).

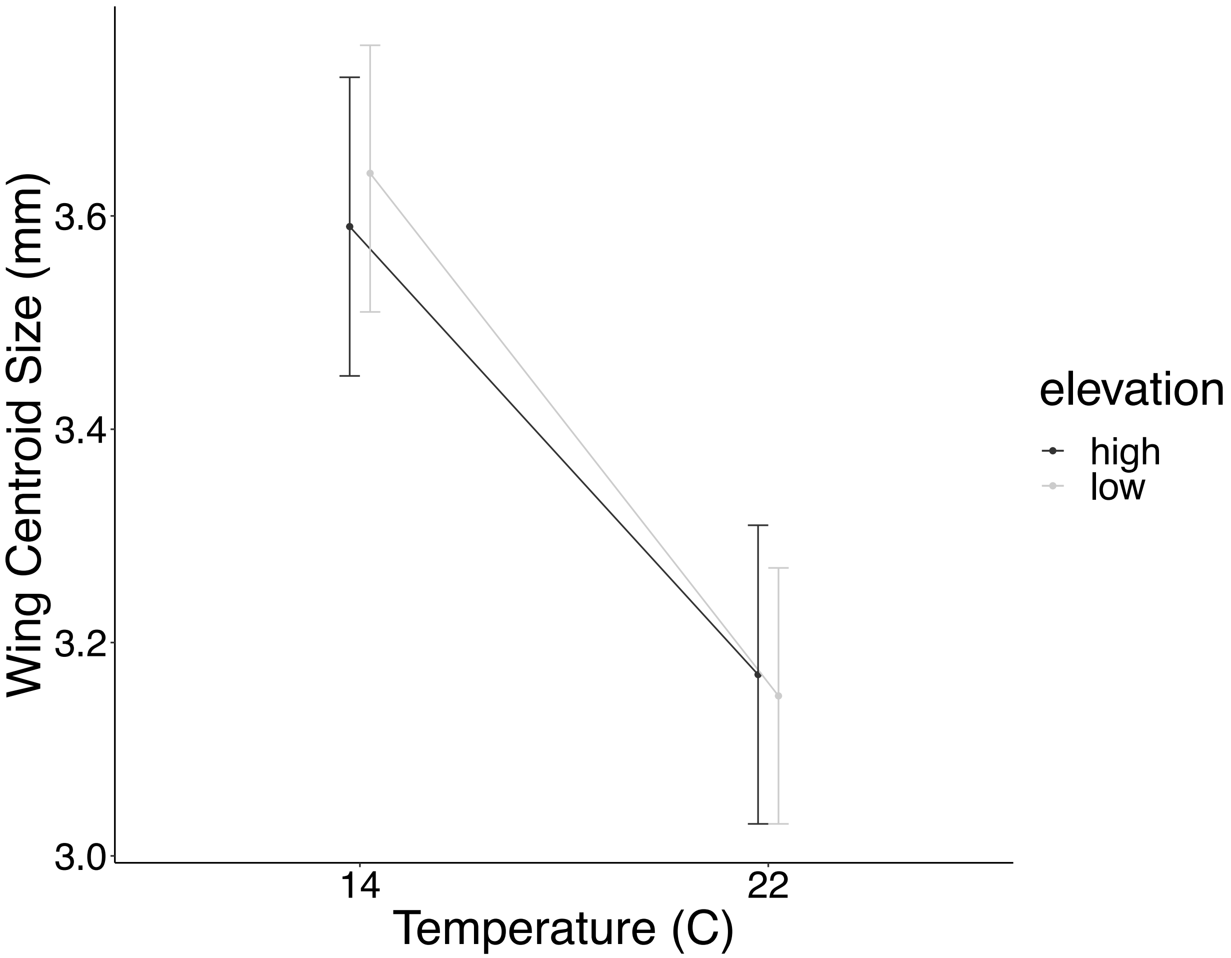

Fig 7b The effects of the reciprocal temperature experiment on the mean centroid size (wing size) of *Drosophila suzukii*. Wing size responded strongly to experimental temperature and we found a significant interactions between experimental temperatures and elevation ($F_{1,1253}=6$; $p<0.05$).

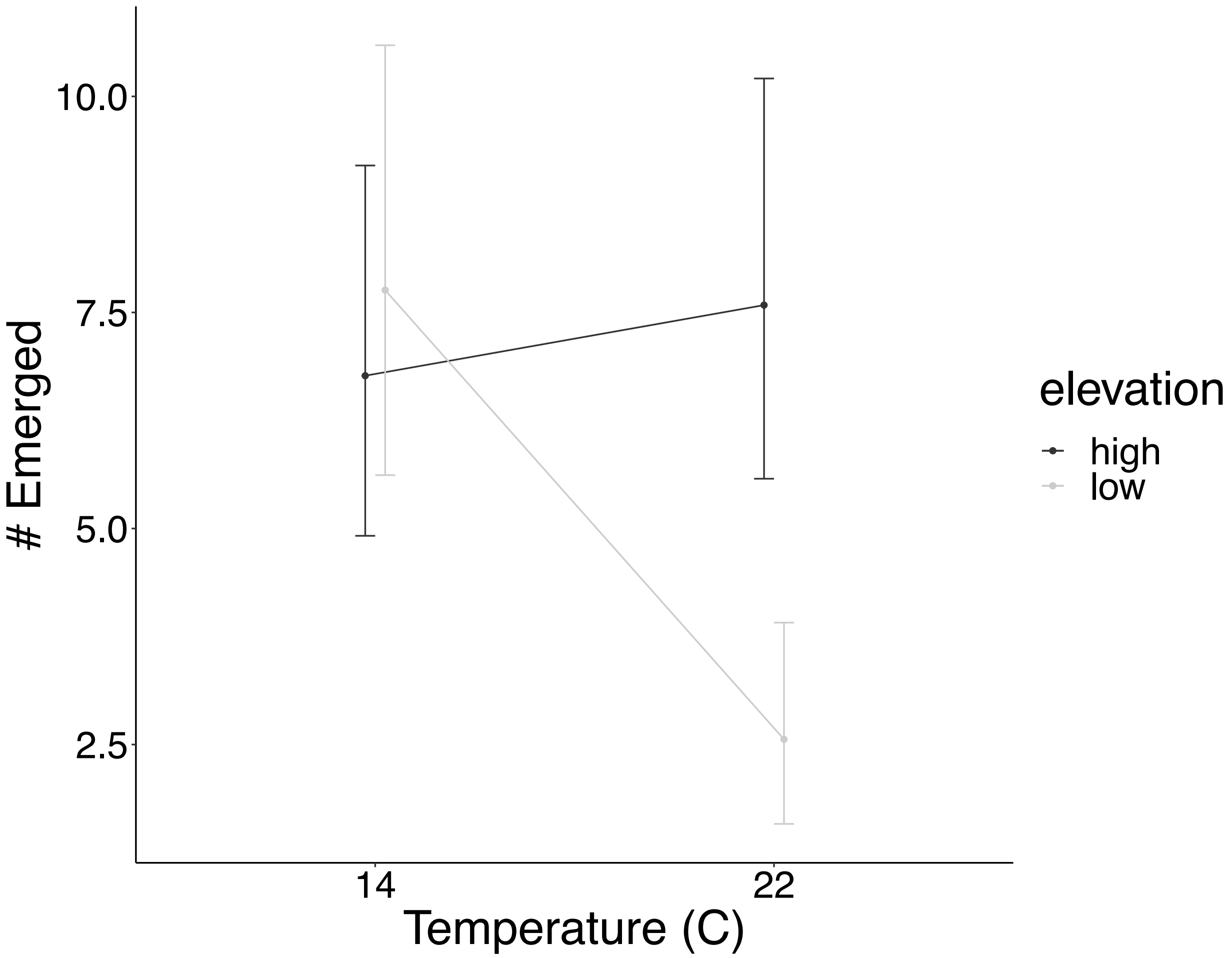

Fig 8 The effects of the reciprocal temperature experiment on the number of flies that emerged of *Drosophila suzukii*. Emergence was influenced by experimental temperature ($\mathcal{X}_1^2$=6; $p<0.05$), elevation ($\mathcal{X}_1^2$=12; $p<0.0001$) and an interaction between experimental temperature and elevation ($\mathcal{X}_1^2$ =59; $p<0.0001$).

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SUPPLERMENTARY MATERIAL

Table S1 Elevations sampled in different months of 2017, and whether or not (y/n) data on abundance and temperature were also collected.

	Elevation							
Month	109	668	1,147	1,523	1,772	2,032	Abundance	Temperature
Jan	X				X	X	y	n
Feb	X			X	X	X	y	n
March	X			X	X	X	y	n
April	X			X	X	X	y	y
May	X			X	X	X	y	y
June	X			X	X	X	y	y
July	X	X	X	X	X	X	y	n
August							n	n
September	X	X	X			X	n	n
October	X	X	X	X	X	X	n	n
November	X	X	X	X	X	X	n	n

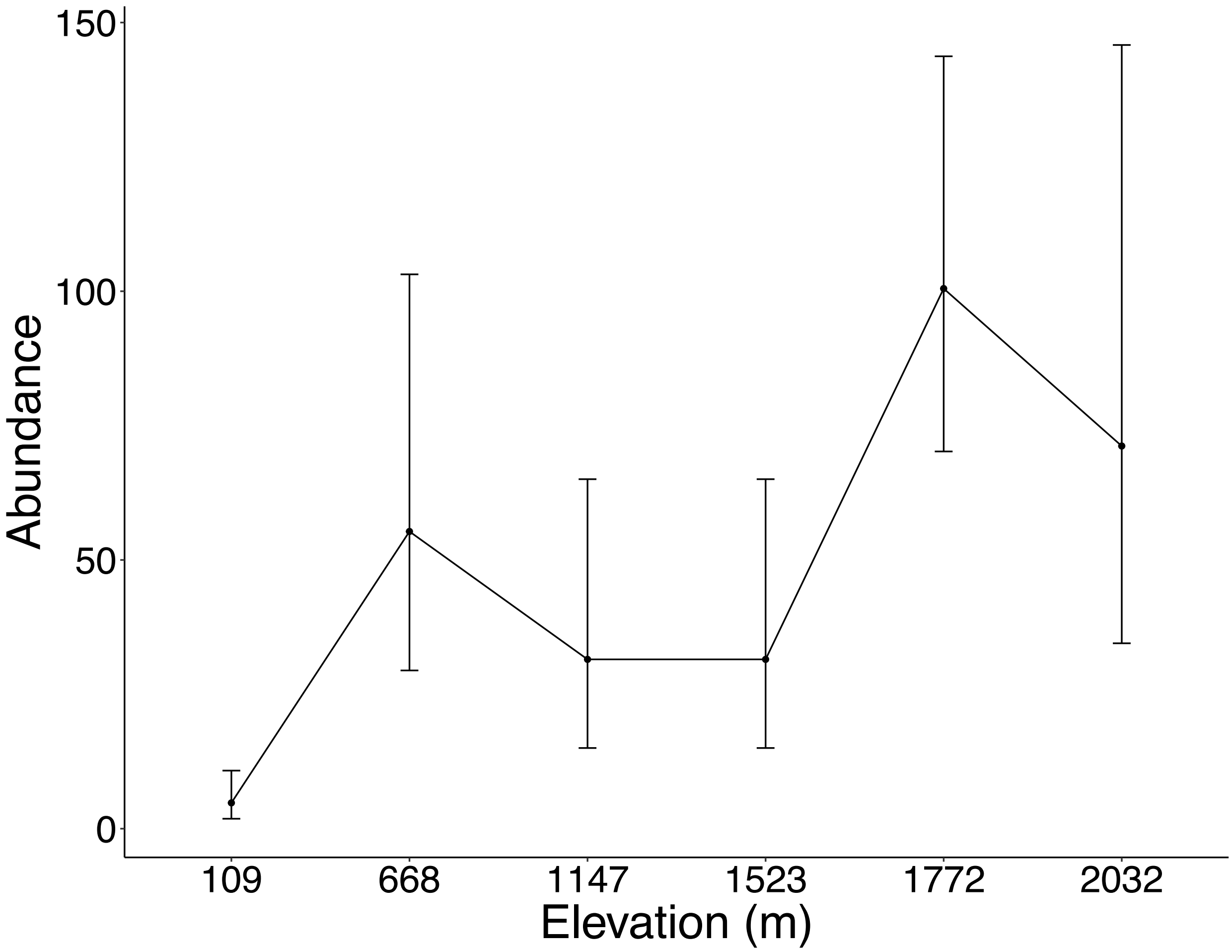

Fig. S1 Relationship between elevation and abundance for July 2017 of *D. suzukii* on Hawaii, showing back-transformed model means and 95% CIs. We found a significant effect of elevation ($F_{6,22}=194; p<0.001$) on the all elevational sites.

GERMAN FOOD PROTOCOL WITH BERRY MEDIA LAYER

German Food Layer

The media utilized in this study consisted of two layers; a thin fruit media layer on top of a regular *Drosophila* media also known as "German food." The German food layer was first created, allowed to set and then a thin layer of the fruit media was layered on top. We typically followed a full recipe, which created 1.5 cm of media and produced approximately 140 standard *Drosophila* vials. The first step in making the first layer is to wear gloves and pre-weigh all necessary materials in preparation for mixing. A dry mix was then created by combining 30g sucrose, 60g glucose, 80g brewer's yeast, 20g yeast extract, 20g peptone, 0.5g magnesium sulfate, and 0.5g calcium chloride in a 1L beaker. The dry ingredients was then mixed well with a stirring rod and place on the stir plate with a mixing bar in preparation for the liquid agar solution. Next we prepared the anti-fungal solution by mixing 1g Nipagin (Methyl 4 Hydroxybenzoate in crystalline form), and 6ml of propionic in a 5ml beaker and set it aside. It was crucial that gloves were worn while making the anti-fungal solution to protect from accidental burns. Next the agar solution was prepared by putting 10g agar in a 2L beaker with add 1L of purified water. The solution was mixed and placed in a not-for-food laboratory microwave and brought to a gentle boil, which took approximately 6 minutes. It was important that we kept an eye out for the solution in the microwave as spills were very difficult to clean. Once the solution have reached a gentle boil, the agar solution was poured onto the dry mixture and slowly incorporated with a stirring rod. The stir plate was then activated, which propelled the stir bar. The mixture was stirred until it cooled to just below 50°C. While the mixture was cooling, 10ml 95% ethanol was added into the antifungal mixture, mixed, then covered as the ethanol was very volatile. Once the mixture on the stir plate was just below 50˚C, the antifungal mixture was incorporated. It was crucial to make sure that the agar mixture was not too hot as it would have deactivated the antifungal solution if added to a hot mixture. Once the anti-fungal mixture was well incorporated, we slowly poured approximately 1-1.5 cm of media into the *Drosophila* tubes. Once the vials were filled it was set aside and we then moved on to creating the fruit layer.

Fruit Media

To create the fruit layer, we first wore gloves and measured out 300 ml of berry puree (we blended fresh strawberries), agar 2.5% solution (600ml water + 15g agar), 30g yeast, 7.5 g yeast extract, 5 ml 95% ethanol, 0.5 g Nipagin (Methyl 4 Hydroxybenzoate in crystalline form), 3 ml propionic acid and cut approximately 140 1cmx3cm strips of porous paper. We first created a 2.5% agar solution by mixing 600ml of water with 15g of agar in a 1L beaker. The mixture was then mixed well microwave until the agar dissolved completely. The agar solution was then placed on the stir plate a long with a stir bar and activated. The agar was stirred until it cooled to just below 50°C. As the agar cooled, 30g of yeast and 7.5g of yeast extract was into the fruit puree and thoroughly mixed. The antifungal mixture was then prepared by mixing of 0.5g nipagin, 3ml propionic acid, and 5ml 95% ethanol and covered. Gloves were worn to prevent burns. Once the agar cooled, the fruit puree mixture and the antifungal mixture was incorporated. Once the mixtures was thoroughly mixed, 0.5 cm of the fruit media was poured on top of the hardened German food media. Once the fruit media was poured into the vials, 1 strip of the porous paper was dropped into the vials for the flies to use as a platform when the media started to get old and sticky. Once the media cooled and hardened, it was then ready to use. For storage, the vials were covered with the appropriate stoppers and place in the refrigerator.