Drosophila it was also shown that mating latency is also affected by body size, age, and diet (Hegde and Krishna, 1997; Somashekar and Krishna 2011; Singh and Sisodia, 2012). In *P. straiata* courtship activities of male and female culminate in copulation (Latha and Krishna, 2014). Longer copulation is an adaptation of males which could reduce the risk of sperm competition with future ejaculations with the help of mating plug which prevents the female from remating before oviposition (Gilchrist and Partridge, 2000). In the present study it was found that flies grown on organic banana fruit had copulated significantly longer compared to flies grown on non-organic banana and wheat cream agar media (Figure 3 and Table 1). Our results in *P. straiata* confirm work of organic banana fruit on reproduction (Chabra *et al*., 2013). Longer the duration of copulation, greater is the transfer of accessory gland proteins and sperm to the mated female (Hegde and Krishna, 1997; Somashekar and Krishna, 2011).

Fecundity is the most obvious trait that influences the reproductive ability of female usually considered as female fitness component. It is known that fecundity is influenced by age, body size, and diet of an organism (Krishna and Hegde 1997). In *P. straiata* flies grown on organic banana fruit based media had greater number of ovarioles compared to flies grown in other two media (Figure 4 and Table 1). In the present study, flies used were of same age and were grown in same conditions but foods were different. Therefore, in the present study quality of food had influenced fecundity in *P. straiata*. Ovariole number was positively correlated with fecundity (Krishna and Hegde, 1997). They also pointed out that greater the ovariole number the greater is the fecundity. Therefore, in the present study ovariole numbers of flies grown on different diets were analyzed in *P. straiata*. Flies grown on organic banana fruit had significantly greater number of ovarioles than those flies grown in conventional banana fruit and wheat cream agar media (Figure 5 and Table 1). Thus these studies in *P. straiata* suggest that organic fruit has positive effect on reproduction**.** Organic banana fruit flies had greater reproductive performance and fitness.

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Female reproductive traits of the model Hawaiian fly *Drosophila grimshawi.*

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Introduction

The evolutionary success of the endemic Hawaiian Drosophilidae, a monophyletic group of ~1,000 species, owes much to the diversity of reproductive strategies of these flies (Kambysellis and Heed, 1971), in conjunction with adaptation of female reproductive function and oviposition behavior to a broad array of breeding substrates and plant hosts (Heed, 1968; Montgomery, 1975; Kambysellis and Craddock, 1997). Members of the well-studied picture wing group of over a hundred species are typically large, long-lived flies

with high fecundity, greater than that observed in other groups of Hawaiian flies. At the outset of cytological analyses of the picture wing group, Hamp Carson arbitrarily chose the species *Drosophila grimshawi* Oldenberg of the *grimshawi* species group as the 'standard' picture wing species (Carson, Clayton, and Stalker, 1967). Polytene chromosome banding sequences of all subsequently studied Hawaiian species were compared to the *D. grimshawi* Standard sequence, and the number and locations of inversions required to derive each sequence from the Standard determined. These inversion relationships were used to derive a chromosomal phylogeny of evolutionary relationships among the studied species (Carson, 1970), long before the advent of molecular phylogenies. In planning the expansion of genome sequencing efforts beyond the model fly *Drosophila melanogaster*, it was natural to select Carson's standard species, *D. grimshawi*, as the representative of the large group of endemic Hawaiian *Drosophila* (*Drosophila* 12 Genomes Consortium, 2007).

D. grimshawi, the genomic model for the Hawaiian *Drosophila*, is somewhat anomalous, however, in that it is not a single-island endemic, which is the characteristic situation for these flies. Originally, this species was thought to occupy all six high islands of the archipelago. The taxon on the youngest island, Hawaii, was then described as a distinct species, *Drosophila pullipes*, by Hardy and Kaneshiro (1972). Subsequently, phylogenetic analyses of the clade indicated that the Kauai and Oahu populations were much more closely related to *D. pullipes* than to those on Maui, Molokai, and Lanai (Piano *et al.*, 1997). These data led to a taxonomic revision which distinguished the Kauai and Oahu populations as a separate but morphologically cryptic species, *Drosophila craddockae* (Kaneshiro and Kambysellis, 1999). The clade thus comprises three closely related species. Importantly, two species, *D. craddockae* and *D. pullipes*, are strict ecological specialists that breed in decaying bark of *Wikstroemia* (family Thymeleacae); *D. grimshawi*, on the other hand, is a generalist that has been reared from ten other families of Hawaiian plants, but not Thymeleacae (Heed, 1968; Montgomery, 1975). The distribution of the species *D. grimshawi* on the three islands of the Maui Nui complex includes four geographically disjunct metapopulations, namely those on the volcanoes of East Maui, West Maui, Molokai, and Lanai. Although currently separate, all islands were connected by land bridges into one composite island, Maui Nui, as recently as 0.3 – 0.4 Myr ago (Carson and Clague, 1995). Despite this, it cannot be assumed that montane forest suitable for *Drosophila* populations extended to the lowland land bridges at times of lower sea level such that the current population isolates were contiguous; nonetheless, the chances for migration and gene exchange among the *D. grimshawi* populations on the separate volcanoes were potentially greater then than at the present. The sequenced genome of *D. grimshawi* is from the East Maui population, specifically from the G1 laboratory stock which was the first successfully established stock of a Hawaiian species. Remarkably, this strain has been in continuous laboratory culture for half a century since the original isofemale was collected from Auwahi, East Maui in 1965.

Now that natural populations are sparse and it is uncommon to encounter this species in the field, the relatively recent collection of several *D. grimshawi* females and establishment of additional laboratory stocks at the University of Hawaii from East Maui, West Maui, and Molokai populations, provided an opportunity to examine ovarian traits and compare them with those of the long established G1 strain. All stocks are isofemale lines, with each derived from a single field-inseminated female. Consequently, all have experienced a population bottleneck. Nonetheless, it is possible that these samples from three disjunct populations display genetic differences in reproductive, behavioral, and other traits, given their current geographic isolation.

Materials and Methods

Besides the G1 stock of *D. grimshawi*, I examined females from four lab strains (ZA6Z2, ZA6Z3, ZA6Z4, and ZA010-5) from a different East Maui site, Makawao Forest Reserve, one strain from north of Hanaula, Waikapu, on West Maui (WM1005), and two strains from a side gulch SE of Kawela Gulch Road on Molokai (MoA, Mo010). Because of the high fecundity of *D. grimshawi*, and its generalist habit, all have readily adapted to the standard laboratory culture protocol for Hawaiian *Drosophila*. This entails supplementation of the Wheeler-Clayton medium for adults and the modified cornmeal medium for larvae with a brew of fermented leaves and stems of *Clermontia* (family Campanulaceae), a common host plant for many Hawaiian species including *D. grimshawi*. Samples of young adult females 4-5 weeks old from each of

the eight strains were withdrawn from the culture jars and sedated by chilling in the freezer for a few minutes, before dissection to remove their ovaries and obtain ovariole counts. Developmental rates in Hawaiian flies are extremely variable (despite uniform nutrition, light-dark cycle, and temperature), such that a proportion of approximately one-month old females still had quite immature previtellogenic ovaries, making accurate determination of ovariole numbers unreliable. Thus, no data were obtained from many dissected flies, which precluded obtaining equal sample sizes for all stocks. The data presented here for Makawao Forest Reserve are based on five females from the ZA6Z2 isoline, six females from ZA6Z3, three females from ZA6Z4, and two females from ZA010-5. The Molokai sample included 11 females from the MoA line, and seven females from the Mo010 stock.

Flies were dissected in chilled Waddington's Ringer solution and the two ovaries placed on separate microscope slides, before removal of the membrane that ensheathes each ovary and teasing apart of individual ovarioles with very fine needles to a point at which I could be sure of a completely accurate count (see Figure 1B). Ovariole counts were made separately for both ovaries of an individual female. In cases where some of the ovarioles already contained mature eggs at their posterior end, the number of mature eggs per ovariole was noted.

Thorax length in mm was used as a proxy for body size. A digital image of a right lateral view of the thorax of each female was acquired, and for calibration a digital image of a slide micrometer, taken at the same magnification. Image J was used to obtain the length from the anterior end of the thorax to the tip of the scutellum. Similarly, a sample of mature eggs dissected from ovaries was imaged and egg length and length of the respiratory filaments determined from the digital images using Image J. Sample means and standard errors of the mean (S.E.M.) of ovariole number per ovary and per fly, and female thorax length, were calculated in Excel for each of the four populations. Comparisons of these traits among populations were made using ANOVA, and between the two East Maui samples via *t*-tests.

Results

Figure 1 shows the morphology of eggs and ovaries of *D. grimshawi*. The most striking feature of the mature chorionated egg of this species is the four long respiratory appendages that are more than twice the length of the egg (Figure 1D). As shown in Figures 1A and C, these elongated respiratory filaments extend anteriorly within the ovariole beyond the germarium region. This is typical of all the Hawaiian bark-breeding picture wing species (Kambysellis and Heed, 1971; Kambysellis, 1993; Kambysellis and Craddock, 1997). Eggs of *D. grimshawi* range in length from 0.88 - 0.91 mm (mean 0.90 $\pm$ 0.01 mm), with respiratory filaments measured at lengths from 1.7 to 2.1 mm. (The filaments are fragile and the tips easily break off during handling; their curved nature also makes their precise measurement somewhat problematic.)

Table 1. Data on mean female thorax lengths (± S.E.M.), and two ovarian traits, numbers of ovarioles per ovary (mean ± S.E.M., and range) and maximum number of mature eggs observed per ovariole, in samples of lab-reared *D. grimshawi* females derived from four field populations in Maui Nui.

Island & Locality	*Isofemale Line(s)*	*Mean Thorax Length (mm)*	*N*[a]	*Mean # Ovarioles per Ovary*	*Range*	*Max # Mature Eggs Observed per Ovariole*
East Maui, Auwahi	G1	2.11 ± 0.07	50	21.2 ± 0.34	16 - 26	(2)
East Maui, Makawao Forest Reserve	ZA6Z2, 3, 4; ZA010-5	2.20 ± 0.07	32	21.8 ± 0.50	15 - 28	(1)
West Maui, Hanaula	WM1005	2.20 ± 0.06	40	22.1 ± 0.51	16 -29	4
Molokai, SE of Kawela Gulch Road	MoA; Mo010	2.19 ± 0.02	36	20.6 ± 0.47	15 - 26	3

[a]Number of ovaries scored to determine ovariole numbers. The number of females measured for thorax length is exactly half this number.

Table 1 presents data on mean thorax length, mean number of ovarioles/ovary, variation in number of ovarioles/ovary, and maximum number of mature eggs per ovariole observed in samples of females from the East Maui G1 strain (the Standard) and seven additional lab strains of *D. grimshawi*, comprising a second recently collected population on East Maui and populations on West Maui and Molokai. Within the species

and within each sample there is considerable variation among individuals in number of ovarioles per ovary, which varies over an almost two-fold range from 15 to 29 (Table 1). Kambysellis and Heed (1971) noted a comparable inter-individual variation in ovariole numbers for samples of field collected flies of other Hawaiian species and attributed this to variable nutrition.

Comparing the Auwahi and Makawao Forest Reserve strains from East Maui, there is no significant difference in female thorax length (t_{39df} = 0.88; P > 0.05) or in number of ovarioles per ovary (t_{80df} = - 0.93, P > 0.05), despite the temporal and geographic difference in the source samples. Moreover, comparing all four *D. grimshawi* populations via a one-way Analysis of Variance, no significant differences were observed in either thorax length (ANOVA, $F_{3,75}$ = 0.56, P = 0.64) or in number of ovarioles per ovary (ANOVA, $F_{3,154}$ = 2.02, P = 0.11). Thus, based on these pilot data, there is no significant genetic differentiation in these two traits among the East Maui, West Maui, and Molokai populations of *D. grimshawi*.

With respect to the maximum number of mature eggs per ovariole, three, or three or four mature eggs per active ovariole, were commonly observed in lab flies from the Molokai and West Maui strains, respectively. The majority of females dissected from the East Maui strains were still young flies (≤ one month old), and therefore either had not yet completed maturation of any eggs, or had at most one mature egg per ovariole. Some of the older females examined in these samples had already oviposited their first batch of eggs and were in the early stages of developing their next batch of eggs, so again the potential maximum number of eggs per ovariole could not be assessed.

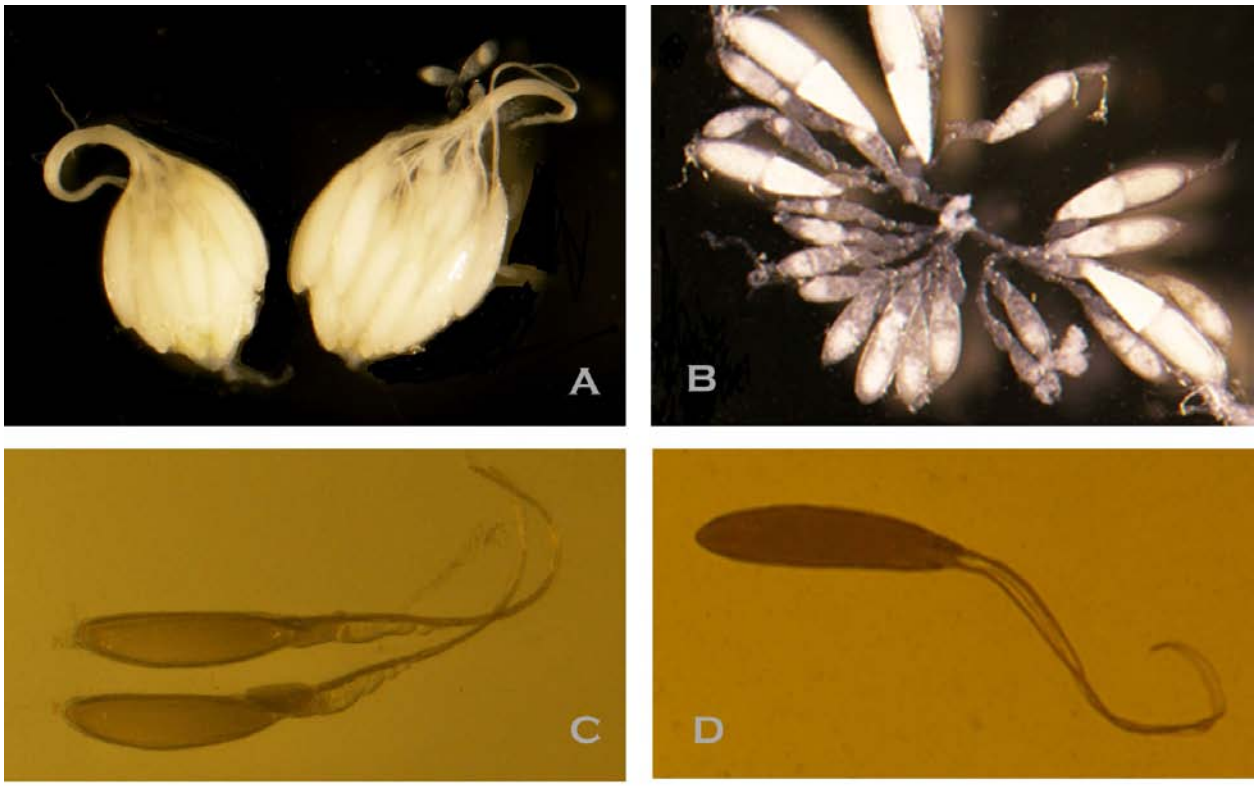

Figure 1. A. A pair of *D. grimshawi* ovaries dissected from a mature female, containing many mature eggs in the most posterior egg chambers. Note that the extremely long respiratory filaments extend to the anterior tip of the germarium and beyond (at top). B. An ovary of a young female teased apart to display the 22 ovarioles which have been left connected at the anterior germarium (center). The most advanced egg chambers are in mid vitellogenesis. C. Two dissected ovarioles each containing one mature egg. Note the younger egg chambers (to the right) lying alongside the long respiratory filaments. D. Mature egg dissected from an ovary showing the four long respiratory filaments at the anterior end of the egg.

Discussion

Based on examination of ovaries of lab-reared flies derived from four different populations of *D. grimshawi* from three islands in the Maui Nui complex, no consistent differentiation was found for a key female reproductive trait, the number of ovarioles per ovary. Combining data from the four geographic populations, the average for the species is 21.4 $\pm$ 0.22 ovarioles per ovary, or 42.8 $\pm$ 0.59 ovarioles per fly. As noted for *D. melanogaster*, ovariole number is a phenotypically plastic trait, subject to both genetic and environmental effects (Wayne *et al.*, 1997). The highest number scored in an individual *D. grimshawi* (from the West Maui isoline) was 55, with 29 ovarioles in one ovary and 26 in the other. The lowest number of ovarioles per fly was 31, with 16 ovarioles in one ovary and 15 in the other. While there is some variation in the number of ovarioles between the left and right ovaries of an individual, it is minimal, generally no more than two or three. It should be noted that the data presented here are for laboratory flies raised on protein-rich media. Kambysellis and Heed (1971) noted that ovariole numbers of lab-reared flies were greater than those of their field-collected parents, presumably because competition in nature for nutritional resources is more intense than in the lab. No ovariole numbers for field-collected *D. grimshawi* were presented in Kambysellis and Heed (1971), as this species is not as common as others in field collections. This is surprising, given that *D. grimshawi* is a generalist with a broad host range with respect to ovipositional resources. It might be assumed that larvae and adults of this species could feed on microbes from an equally broad array of plant resources, and would therefore be able to sustain larger populations than those of ecologically specialized species that are limited by the rarity of their host plants. On the other hand, as a generalist, *D. grimshawi* competes with many other sympatric *Drosophila* species using the same breeding resources, and this factor may limit population sizes in the field.

It should be noted that the value of 28 ovarioles/fly for *D. grimshawi* published in Markow and O'Grady (2007) is erroneous. No standard errors or sample sizes are provided, so the source of this value is unclear. In this species it is misleading to simply count the number of mature eggs observed in a female as an indicator of the number of ovarioles, since ovariole function in *D. grimshawi* is asynchronous.

Wayne *et al.* (1997) estimated the mean number of ovarioles per ovary for *D. melanogaster* at 15.1 from a sample of 1152 ovaries. Thus, ovariole numbers in *D. grimshawi* (21.4 per ovary) are higher than those in *D. melanogaster* (David, 1970; Wayne *et al*., 1997), but the difference is not as great as the dramatic difference in body size between these two species. It is important to note, however, that ovariole number and thorax length are not genetically correlated (Telonis-Scott *et al.,* 2005), although the two traits may be environmentally correlated. Given that *D. grimshawi* is the largest of the 12 *Drosophila* species for which full genome sequences are available, it is not surprising that its eggs are significantly larger than those of the other 11 species (Markow *et al.*, 2009). *D. grimshawi* eggs are twice as long as those of *D. melanogaster*, and twice the weight, and have an outer endochorion that is eight times as thick (Margaritis *et al.*, 1983). In addition, there are four rather than two respiratory appendages at the anterior end of eggs of *D. grimshawi* that are extraordinarily long. For further details of the ultrastructure of the *D. grimshawi* chorion, the reader is referred to the scanning electron micrographs presented in Margaritis *et al.* (1983), Kambysellis (1993), and Figure 1 of Piano *et al*. (1997), along with the accompanying descriptions.

Ovariole number is a fitness-related trait that shows genetic variation within species but is under relatively strong stabilizing selection (Wayne and Mackay, 1998). But this trait is just one component of the extremely high potential fecundity of *D. grimshawi* compared to *D. melanogaster.* In common with many other members of the *grimshawi* and *planitibia* species groups, the eggs and ovaries of *D. grimshawi* fit into reproductive type IIIb, one of the seven discrete reproductive types recognized among the Hawaiian species (Kambysellis and Craddock, 1997), and the one with the highest fecundity of all among the diverse array of reproductive strategies in the endemic Hawaiian *Drosophila*. Besides high ovariole numbers, this reproductive type is characterized by development of up to 4 mature eggs per ovariole. In fact, the maximum number observed in the sample of females from the eight lab stocks of *D. grimshawi* examined in this study was four mature eggs per ovariole. One other factor besides the ovarian structure and function of *D. grimshawi* is their exceptionally long reproductive lifetimes. Although no systematic attempts have been made to collect data on adult longevities, it is known that under optimal conditions many picture wing species can survive in the lab for over a year. Moreover, lab females can continue to mature and lay eggs. Carson *et al.* (1970) reported that

a field-captured inseminated female of *D. grimshawi* continued to produce fertile eggs for almost a year without additional insemination. This example emphasizes the abundance of sperm that can be stored and remain fertile for many months in the spermathecae and ventral receptacle of this species, as well as the enormous numbers of eggs produced per female over her long reproductive life. At any point in time, a mature female of *D. grimshawi* can potentially carry an egg load of 100 or so mature eggs in her ovaries (Craddock and Kambysellis, 1997). For comparative purposes, the egg load parameter (the number of ovarioles per fly times the number of mature eggs per ovariole) provides only a rough measure of potential female fecundity, given the asynchronous nature of ovariole function in these Hawaiian picture wing species and the lack of solid data on reproductive longevities. Of course, realized fecundity is typically less than potential fecundity. By all measures, however, the potential lifetime fecundity of *D. grimshawi* far exceeds that of nonHawaiian species and in particular, that of *D. melanogaster* and the other ten *Drosophila* species with complete genome sequences. The availability of these sequence data now provides the chance to address many important questions about the molecular basis of evolutionary differences in longevity, reproductive, developmental, and other traits within the genus *Drosophila.*

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Drosophila suzukii has been found in tropical Atlantic Rainforest in southeastern Brazil.

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Drosophila suzukii (Matsumura, 1931) belongs to the *Drosophila melanogaster* species group, probably native to the southeastern Palaearctic region (Bächli, 2013). Its ability to feed and breed in healthy fruits led it to become an agricultural pest. *D. suzukii* is an invasive species, being recorded in North America