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The role of body size on the outcome of mating interactions in Drosophila melanogaster

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by

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Abstract

Sexual selection is the process by which some individuals produce more and/or better quality offspring than others because they are better at securing mates. While this may be accomplished by defeating same-sex rivals (intrasexual selection), individuals of one sex (typically females) may also "decide" on the suitability of individuals of the opposite sex (typically males), resulting in intersexual selection on attractive traits. While a great deal of scrutiny has focused on how sexual selection influences male display traits, much less scrutiny has been directed toward the factors underlying female preference, including genetic variation, as well as the extent to which both sexes are involved in mate choice.

In *Drosophila melanogaster*, a model species for the study of sexual selection, previous studies have examined the role of body size variation in a single sex on the behaviours and outcomes related to courtship and copulation. However, few studies have simultaneously varied both male and female body size. In my first study (Chapter 2), I experimentally paired male and female flies from across a wide spectrum of body size phenotypes and quantified several behavioural traits: time to courtship initiation, length of courtship and length of copulation. I found that absolute body size differences affected length of courtship and that relative body size differences affected time to courtship initiation.

While Chapter 2 demonstrated how mate choice may be expressed within a single generation of individuals, whether individual preference variation in females had a genetic component had yet to be determined experimentally. In my second study (Chapter 3), I investigated if female body size preference had a genetic component by

directly selecting on female preference over multiple generations. Using artificial selection, I “penalized” females that mated with males of certain body sizes over 20 generations and observed several significant differences in female preference behaviour. In all treatments, females tended to associate significantly more with males of body sizes different from those they were artificially selected against.

These results not only suggest that body size in both sexes can significantly influence female preference behaviours, but that body size may be a trait possessing significant genetic variation with the potential to be strongly shaped by sexual selection.

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Table of Contents

List of Tables

List of Figures

1.0 General Introduction

1.1 Sexual Selection

Sexual selection, the differential success of individuals competing for mates, is one of the driving forces behind speciation and the evolution of exaggerated male traits in many species (Andersson, 1994). Some extravagant male features that otherwise might seem maladaptive acquire meaningful function when considered in the context of female choice and/or male-male competition. Sexual selection results from competition for acquiring mates, with unsuccessful competitors siring fewer or no offspring in comparison to more successful individuals. Thus, individuals who successfully mate will have their genetic material propagated into the subsequent generation. Darwin (1859) noted that competition for mates is not directly a struggle for survival, and that traits that are sexually selected for may not be naturally selected for, and vice versa. While many traits can be both naturally and sexually selected, such as general metabolic efficiency or pathogen resistance (Andersson, 1994), some traits evolve for a narrower purpose. For example, the large elaborate, twisted antlers of male white-tailed deer (*Odocoileus virginianus)* may have the deleterious effect of being cumbersome or getting it caught in underbrush, but serve as weapons in skirmishes with other conspecific males, with the loser potentially not being able to mate at all (Gadgil, 1972). This form of selection, called intrasexual selection, addresses interactions within a single sex, wherein the "fittest" individuals have the greatest chance of copulating and siring offspring. In contrast to male white-tailed deer antlers, the bright, decorative plumage of male peacocks (*Pavo cristatus*) does not aid in physical skirmishes with rival males, but is instead used as a display for directly courting females, allowing a female to potentially choose whom she prefers (Loyau *et al*., 2005). This form of selection, called intersexual

selection, primarily involves mate choice competition in the form of courting rituals, elaborate male ornamentation, and other species-specific indicators of fitness (Andersson, 1994).

Males, in most species, are the sex that actively court females in order to produce offspring. Fundamentally, the stronger sexual selection acting on males arises from anisogamy: the propensity for females to produce large, energy-rich gametes and for males to produce small, highly mobile gametes (Andersson, 1994). In addition, energy differences required for females to produce a limited number of energy-rich ova when compared to a male's small, motile sperm, compounded with the large energetic demands of pregnancy, generally make females of most species highly discriminatory in their choice of mating partner (Andersson, 1994). For example, in the fruit fly *Drosophila melanogaster*, fecundity is limited primarily by a female's ability to produce eggs, whereas male fitness is only limited by the number of females that he is able to inseminate (Bateman, 1948). Thus, the ability of an individual to discriminate which potential mate has "good genes", or can provide the greatest direct benefits, is vital for maximizing an individual female's lifetime reproductive success or that of her offspring (Bateson, 1983).

1.2 Body Size as a Sexually Selected Trait

One trait that can result in one individual being selected over another is body size (or traits strongly associated with body size). For example, male broad-headed skinks (*Eumeces laticeps*) of larger than average body size are more commonly found copulating with females during their breeding season than smaller males. As large males tend to chase off small males that attempt to approach females, this might be a trait strongly

shaped by intra-sexual selection (Cooper and Vitt, 1993). However, when no-choice assays (involving a single male and female) were conducted in the laboratory, removing any confounding effects of male-male competition, females actively rejected a significantly larger proportion of smaller males than larger males, suggesting that body size is also subject to intersexual selection. Since male broad-headed skinks do not seem to provide any obvious direct benefits to females, females mating with larger males may be selecting them on the basis of acquiring indirect benefits, which consequently results in sexual selection on body size (Cooper and Vitt, 1993). Comparatively, in Mottled Sculpins (*Cottus bairdi*), males are tasked with guarding a female's eggs once they have been fertilized. As larger males are more effective at guarding eggs against predators than smaller males are (due to their increased size), body size is again sexually selected for, as larger male body size directly benefits female fitness (Brown, 1981). Mate choice based on body size is also seen in *D. melanogaster*. Females have been observed to prefer larger bodied males, though whether female discrimination among potential mates is specifically due to body size or a correlated trait is still unknown. While some studies have claimed the mating advantage of larger males is a "purely male effect, with no involvement of female choice" (Partridge *et al*., 1987a; Partridge *et al.,* 1987b; Wilkinson, 1987), others have suggested that females actively exercise mate choice (Markow, 1987; Pitnick, 1991). Males also have been observed to demonstrate mate choice favouring larger females over smaller females (Byrne and Rice, 2006), as the former are typically more fecund than the latter (Robertson, 1957). Additionally, males strategically adjust their ejaculate size based on female body size, delivering more sperm to larger females than to smaller females (Lupold *et al.*, 2010). While body size has been

demonstrated to be a trait strongly subject to sexual selection in this species, preference for body size may not be uniform in a population. Since the optimal male strategy may be to mate non-discriminately with as many females as possible (Andersson, 1994), male preferences could be more broadly examined as though they were uniform in a population. However, optimal female strategy is thought to be more conservative and cautious with respect to selecting a potential mate (Andersson, 1994). Thus, variation in female preferences within a population requires careful study to better understand how sexual selection may influence body size.

1.3 Variation in Mate Preference for Body Size

Variation in female preference is an important aspect of sexual selection that has received a relatively small amount of attention in *D. melanogaster*. This is partly due to the difficulty of measuring female preference, as more than one source of selection may be acting on female preference at any given time (Wagner, 1998). Additionally, during assays, females are commonly presented simultaneously with more than one stimulus, with preference information inferred based on subsequent female choice. While such results are relatively easy to interpret, they are not useful for directly measuring preference, as preference is one of many factors that determine mate choice. Thus, to understand if (and how) selection can act on female preferences, rather than on how female preference may result in selection on male traits, indirect measurements of preference functions are not adequate: direct quantification of individual female preferences is required. While past studies have examined intrasexual competition on sexual selection in females (Rosvall, 2011) and costs of antagonistic male persistence toward sexually attractive, high fitness females (Friberg and Arnqvist, 2003), there have

been few studies that have focused on how variation in female mate choice may be related to both male and female body size characteristics.

Previously, Lefranc and Bundgaard (2000) showed that smaller *D. melanogaster* females copulate longer than either medium or large-bodied females, with small females having least fecundity. While three size classes of male and female flies were used in the experiment, the experimental population used, Oregon-R, is a highly inbred fly stock possessing limited genetic variation. As such, other outbred populations may not behave similarly. Friberg and Arnqvist (2003) demonstrated that *D. melanogaster* females had shorter times to copulation with large males than small males, but suffered negative fitness consequences when mated to these "preferred males" compared to when they were mated to small or intermediately sized males. However, in this experiment, only two size classes of males were used, with no control over female body size, greatly limiting the extent of behaviours that might be explained by body size variation. Furthermore, variation in larval food quality was used to generate male body size variation in their study, an approach that has been shown to affect immunity (Fellous and Lazzaro, 2010) and potentially affect male mating success (Valtonen and Rantala, 2011). While these studies and others like them (e.g. Spieth, 1952; Fulker, 1966; Partridge *et al*., 1987a) have laid the foundation of our current understanding of how body size and mate preference are related, I am aware of only one study (Turiegano *et al*., 2012) that has investigated the relationship of co-varying male and female body size on mating behaviour. Though Turiegano *et al*. (2012) did find that both male and female size contributed to some differences in pre- and post-copulatory mating variables, their study too may have some potential limitations. They used a highly inbred stock of flies (Canton-S), which may

have limited the amount of genetic variation present in both males and females. Additionally, the flies used in their experiments were not cultured under their standard culture conditions, which had the potential to alter multiple aspects of behaviours related to mating (Ribó *et al.,* 1989; Cotton *et al.,* 2006; Long *et al.,* 2009). While Turiegano *et al'*s (2012) study provided a much needed examination of how both male and female body size variation influence mating behaviours, further studies are required to provide broader and more accurate insight into *D. melanogaster* mating behaviours.

In Chapter 2 of my thesis, I investigate how body size variation in both male and female *D. melanogaster* influence mating behaviour. Using a large, outbred, wild-type population of fruit flies, I experimentally paired male and female flies from across a wide spectrum of body size phenotypes using both an absolute and relative body size classification scheme, quantifying a number of behavioural traits expected to vary with body size. If we find that variation in both male and female body size results in corresponding variation in mating behaviours, subsequent investigations examining mate choice should consider that variation in both male and female body size can potentially have a significant effect on observed behaviour.

1.4 Experimental Evolution of Female Preference

While Chapter 2 examined variation in female mating preferences, the sources of said variation are still relatively unknown. Some of the variation in mate-choice decisions is non-heritable: time and energy costs of mate sampling, increased risk of predation, variable territory and resource quality, and abiotic factors such as temperature and opportunity for concealment can all reflect local environmental and geographical variation (Jennions and Petrie, 1997). However, most theoretical models assume a

heritable genetic basis to mating preferences. Indeed, additive genetic variation for mating preferences have been observed in a wide range of species (Jennions and Petrie, 1997), including *D. melanogaster* (Andersson, 1994). Thus, examination of population-wide preference for a given trait may provide an incomplete view of which factors may be influencing preference evolution (Wagner, 1998). If preference can vary adaptively between individuals (i.e. based on local environment), a female may benefit from having preferences that differ from those of the population on average (Garland and Rose, 2009). One way we can examine the genetic basis for variation in female preferences is by using experimental evolution. Experimental evolution is an approach that allows investigation of how traits respond directly to sexual selection (Garland and Rose, 2009). By studying replicate populations over multiple generations under standardized and replicable conditions, co-evolution of male traits and female preferences for those traits can be quantitatively modeled. Experimental evolution of female mating preferences was examined by Wilkinson and Reillo (1994) using stalk-eyed flies (*Cyrtodiopsis dalmanni*) to study female choice in response to artificial selection on eye span length. *C. dalmanni* exhibit sexual dimorphism in eye span, a heritable trait, with males showing a steeper allometric relationship of eye span to body size compared to females. When control flies (no selection) were compared to flies from populations subjected to 13 generations of bidirectional artificial selection (for long eye span or short eye span), females from both the long eye-span treatment and the control treatment preferred males with long eye span. Females from the short eye span treatment, however, preferred males with short eye span, suggesting a genetic correlation between female preference and the sexually selected male trait (Wilkinson and Reillo, 1994). While Wilkinson and Reillo's experiment

showed that a heritable male trait may be artificially selected for to measure correlated responses in female preferences, the experiment was limited by not directly measuring female preference evolution; female preferences were indirectly quantified respective to differences in male eye span width, as female preference itself was not subject to artificial selection.

Additional work on guppies (*Poecilia reticulata)* has also demonstrated the viability of experimental evolution for investigating female mate preferences. Houde (1994) artificially selected on a male guppy display trait (orange colouration) over 3 generations to determine if a genetic correlation existed between it and female preference for that trait. If a correlation existed, artificially selecting for increased or decreased amounts of orange colour in males was expected to result in a corresponding shift in female mating preference. In this study females from the treatment in which males were selected for increased colouration showed stronger preference for orange than females from the treatment selecting for decreased colouration. While this study demonstrated preference evolution as a result of artificial selection in only three generations, Houde noted that divergence in preference between treatments decreased or disappeared in the third generation. This may have been related to the low sample size used in the study (N = 6 males per test group), with population density high enough so that male-male interference reduced female ability to exercise preference (Houde, 1994). The breakdown in genetic correlation under laboratory conditions could potentially be addressed by more closely mimicking natural population sizes (i.e. increasing sample size) and/or extending the duration of the study to increase the number of generations; however, it has been suggested that even if populations are maintained in large numbers,

numbers may not be sufficient to maintain significant genetic variation in captive populations (Briscoe *et al*., 1992). More recently, Hall *et al*. (2004) used artificial selection on *P. reticulata* to better understand how male attractiveness and female mate choice respond over 3 generations. Using partitioned aquariums, individual virgin females could observe up to 5 males, with number of "visits" to each male recorded and used as a measure of male attractiveness, while female choice consistency between-females was used as measure of her preference for attractive males. Surprisingly, subsequent generations within each artificial selection treatment (selecting up on male attractiveness, selecting down on male attractiveness, selecting up on female preference for attractive males, and a control) revealed no significant response to direct selection. As direct and indirect selection are expected to cause significant evolutionary changes, the study's low statistical power may partially explain why they observed no response. Hall *et al.* (2004) note that a lack of additive genetic variation is an unlikely explanation for the lack of observed selection, as previous studies using *P. reticulata* had comparable selection intensities that reported significant changes in male colouration. That Hall *et al*'s (2004) artificial selection approach failed to report a response became a primary motivator for Chapter 3 of my thesis, using artificial selection to determine if female *D. melanogaster* possess significant genetic variation for mate preference with respect to an attractive male trait (body size).

Experimental evolution has been previously used to investigate body size in *D. melanogaster* (Huey *et al*., 1991; Partridge and Fowler, 1993; Partridge *et al*., 1998; Turner *et al*., 2011), a trait with considerable genetic variation (Alpatov, 1930; Gockel *et al.*, 2002). However, to the best of my knowledge, no past studies using experimental

evolution have examined female preference evolution for male body size in this species. In Chapter 3 of this thesis, I directly examine the evolution of female preference for body size by imposing artificial selection on females for 20 generations. I have also improved upon the methodology used in previous studies by utilizing 3 size classes of *D. melanogaster*, which captures a wide phenotypic range of body sizes. Additionally, I use two wild-type populations of flies (IV and LHm) to help control for potential population-specific preference bias that may have been present in a given fly stock, which allowed me to achieve a measure of consistency and control in identifying adaptive female mate choice that has not yet been achieved in experimental evolution studies using *D. melanogaster*. Together, the improvements used in this experiment will help to better our understanding of both the evolution and mechanisms of female preference for body size in *D. melanogaster*.

1.5 Conclusions

Chapter 2 and Chapter 3 of this thesis are an attempt to expand our knowledge on how body size influences multiple aspects of *D. melanogaster* mating behaviour. Together my results of how body size variation affects mating behaviour and the extent to which female preference variation for body size can be directly selected upon will enable future studies expanding on these topics to better contextualize their own results. More generally, my studies contribute to the ever-expanding fields of evolutionary biology and behavioural genetics as we attempt to more broadly understand how preference-specific traits can be shaped through sexual selection.

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2.0 The role of absolute and relative body size of males and females on mating behaviour in *Drosophila melanogaster*

2.0.1 Abstract

In *Drosophila melanogaster*, a model species for the study of sexual selection, previous studies have examined the role of body size variation in one sex on the behaviours and outcomes related to courtship and copulation, but there have been few studies that have simultaneously varied both male and female body size. In this study, I experimentally paired flies from across a wide spectrum of body sizes phenotypes and quantified a number of behavioural traits: time to courtship initiation, length of courtship and length of copulation. I found that absolute body size affected length of courtship and that relative body size affected time to courtship initiation. This study reveals how the outcomes of interactions between the sexes often depend on the specific phenotype of both sexes.

2.1 Introduction

Sexual selection, the differential reproductive success of individuals, is one of the driving forces of evolutionary change and speciation (Andersson, 1994) and is thought to be primarily responsible for the evolution of weaponry and exaggerated displays in males and preferences for display traits in females in many species (Andersson, 1994). Darwin (1859) first noted that competition for mates is not directly a struggle for survival, but instead to reproduce; sexually selected traits tend toward fitness optima in much the same manner that natural selected traits do, with their development affecting the life-histories of entire species. While a variety of morphological, physiological, and behavioural traits have become modified or exaggerated in response to sexual selection, body size is recognized as a trait that is frequently subject to the effects of sexual selection in a wide range of species (Andersson, 1994). In most invertebrates and poikilothermic vertebrates, females are frequently the larger sex for a variety of reasons, including an increased direct fitness benefit (higher potential fecundity), an increased need to provide parental resources/care, and for the ability to withstand harassment suffered from males during courtship and copulation (e.g. Esperk *et al.,* 2007). However, variation in body size also plays an important role in male-male competition for access to females. For example, male fig wasps (*Sycoscapter sycoscapter*) fight over mating opportunities until one of the males is exhausted and retreats. Larger male fig wasps have a distinct fighting advantage over smaller males, as body size is positively correlated with endurance, resulting in relatively larger males gaining preferential access to females (Moore *et al.,* 2008). Body size also plays a role in intersexual selection. In dance flies (*Empis borealis*), males carry a nuptial "gift" of insect prey, which is fed upon by the female during copulation. Larger

males are able to carry heavier nuptial gifts, resulting in prolonged copulation duration and subsequently a larger volume of sperm that is transferred (Svensson and Peterson, 1987). Despite the importance of body size as a sexually selected trait in mate choice, measurement of inherent female mating preferences with regard to varying male body size has yet to receive much attention (Wagner, 1998). One reason for this may be that experimental designs frequently used for measuring female preferences typically present two males to one female, with preference information then derived from a female's choice between stimuli. However, this approach introduces the potential confounding effect of male-male competition; males often exhibit plastic behavioural responses when encountering rivals. For example, in fruit flies (*Drosophila melanogaster)*, males copulate longer if there is another male present than if they are alone (Zhang *et al.*, 2006; Bretman *et al.*, 2011). Alternatively, the use of "no-choice" experimental designs may allow for less ambiguous measurements of inherent female preferences to be made, without the confounding effects of male-male competition or social modulation (i.e. when more than two individuals are present) affecting behaviour. While single male-female interactions outside of a group may be unlikely in certain species, knowing how female mating preference is influenced by body size absent confounding influences remains of fundamental importance to understanding how sexual selection may act on whole populations. Furthermore, no-choice results can be used to help formulate realistic null hypotheses with which to compare a situation where an organism has a choice (Olabarria *et al*., 2002).

In *D. melanogaster*, mate preference based on male body size has been shown to be an important factor in several aspects of pre- and post-copulatory behaviour. Larger

males have been shown to have shorter times to copulation initiation (Partridge *et al.*, 1987b), are more fecund than smaller males (Friberg and Arnqvist, 2003, but see Pitnick, 1991), and transfer more sperm during copulation than smaller males (Lupold *et al.*, 2011). Females that mated with these larger males, however, suffered greater lifetime direct fitness consequences compared to those mated to smaller males due to a combination of physical harm incurred during courtship/copulation and the toxic side-effects of the accessory proteins present in seminal fluid (Pitnick, 1991; Friberg and Arnqvist, 2003; Long *et al.*, 2010). Since *D. melanogaster* females can actively reject "unwanted" male copulation attempts (Spieth, 1952; Dickson, 2008), the observation that larger males copulate more quickly with females than smaller males may suggest female preference for this trait (Fulker, 1966). However, the advantages that larger males had in obtaining shorter times to copulation may have been primarily due to their increased physical abilities in coupling with females, especially with smaller females having a decreased ability to resist mating (Partridge *et al.*, 1987a; Turiegano *et al.*, 2012). Whether it is the size advantage of larger males, female preference for larger males, or a combination of the two that result in the observed shorter times to copulation remains unclear. As past studies have typically varied body size within a single sex, investigating the potential interaction between female mating preferences and male body size is difficult, as different body sizes as perceived by each sex may affect the overall response observed for a particular mating behaviour.

While many studies have examined mating behaviour in *D. melanogaster*, the effect of both co-varied male and female body size on sexual behaviour has received relatively little attention, with the notable exception of recent work by Turiegano *et al.*

(2012). In that study the authors found that male size, female size, and their interaction had a significant effect on the length of copulation latency (where length was negatively correlated with male size and positively correlated with female size), courtship latency depended on the female-male size difference (length was negatively correlated with male size and positively with difference in size). The number of wing extensions by males was positively correlated with female size, and time to first female movement was positively correlated with both male and female size. While this study provided much needed insight into male/female mating dynamics, the study had some potential limitations. These included the use of a population of flies (Canton-S) with a history of inbreeding, which may not possess the typical amount of genetic variation present in other populations (see Rice *et al.,* 2005). Furthermore, the developmental environment of the experimental Canton-S flies used was not typical of their normal culture conditions. Flies obtained in their experiment were reared at low population density, which may have allowed greater access to resources than females would typically have (under standard culture conditions) and had the potential to alter multiple aspects of behavior, including mate preference (Ribó *et al.,* 1989; Cotton *et al.,* 2006; Long *et al.,* 2009). In addition, the range of body sizes obtained by Turiegano *et al*. (2012) for both male and female flies may not reflect the range of body size variation typically present in the flies' population. While their method of quantifying mean body size for each sex was valid (using wing length as a proxy), the amount of biologically meaningful body size variation present *within* each sex was not discussed or reported. As phenotypic extremes in body size are rare, my study improved upon comparing body size variation both within and between

sexes by using discrete blocks of body size measurements and arranging interactions in a multi-factorial scheme.

Here, I investigate the effects of female choice on mating behaviour and the potential interaction between female and male body size variation in both male and female *D. melanogaster* on i) latency to courtship initiation, ii) length of courtship, iii) length of copulation , and iv) incidence of successful courtship. These behaviours were specifically chosen because they reflect *D. melanogaster* mating behaviors that are predicted to vary with body size (Partridge *et al.*, 1987a; Lefranc and Bundgaard, 2000; Long *et al.*, 2010). Firstly, I predicted that larger-bodied females would be initially courted faster than smaller-bodied females regardless of male body size. Secondly, I predicted that larger-bodied females would require longer courtship times than smaller-bodied females before copulation was initiated, with courtship time decreasing as male body size increased. Finally, I predicted that larger-bodied females would copulate longer than smaller-bodied females regardless of male body size. An additional consideration for the study of male and female interactions is that individual *D. melanogaster* may not perceive their mate's body size in an absolute sense; by also considering *relative* body size variation with respect to pre- and post-copulatory mating variables, new insights into the interactions between male and female body size may be revealed. In this study I used both an absolute and a relative body size classification scheme to further investigate if body size variation significantly influences *D. melanogaster* mating behaviours.

2.2 Materials and Methods

To examine the effects of body size variation on mating behaviour, adult virgins were collected from the *Ives* (IV) base stock, a large (N ~ 2800 adults/generation),

outbred, wild-type population originally founded from a sample of *D. melanogaster* collected in South Amherst, Massachusetts, USA in 1975 that has been maintained in laboratory for >900 generations (Rose, 1984). Flies were reared at a population density typical of their normal culture conditions at 100-120 individuals/vial (Mallet and Chippindale, 2011) on standard banana/molasses/corn syrup/killed-yeast media (Rose, 1984). IV populations were maintained on a 14 day culture cycle, with all flies housed in a humidity-controlled incubator on a 12 hour light/dark diurnal cycle at 25 °C.

From this population, newly eclosed virgin adult males and females were collected every 6-8 hours starting on the 9th day of their culture cycle. These flies were then sorted by size using a Performer III model SS-3 sieve shaker (Gilson Company Inc.) (Long *et al*., 2009). The sieve shaker mechanically separates flies along a column of successive sieves, each with holes 5% larger in diameter than the sieve below it (diameter of top-most sieve holes = 1420 µm, diameter of bottom-most sieve holes = 998 µm). Flies were placed in the top sieve of the column under light CO_2 anesthesia, the shaker was activated at a rate of 3600 vibrations min^{-1} for two minutes, facilitating the flies' downward movement. This approach was used instead of a traditional approach to varying body size by larval crowding (obtaining variation in body size by increasing egg density to promote greater amounts of larval competition) or varying larval food quality methodologies (lowering the nutrient density of food to obtain variation in body size) because the body size variation obtained under normal culture conditions more accurately reflects that which exists in the entire population (Rice *et al.*, 2005). Once sorted, flies were lightly anesthetized and sorted by sex. Sorted flies used were housed in same-sex vials containing food for 24 h to allow flies to recover from the CO_2 anesthetic. Four

body size categories were designated for females (small, medium, large, and extra-large), and three size classes for males (small, medium, and large) that cover the complete range of phenotypic variation for body size present in this population. Together, these categories result in 12 possible combinations of males and females using absolute body size and 3 treatment combinations using relative body size (see Table 1). Arena chambers for observing male and female interaction were constructed using plastic weigh boats (41mm x 41mm x 8mm), covered by a transparent plastic sheet (44mm x 44mm) to prevent the flies' escape (Figure 1). In each arena, a small amount of live yeast was added to satisfy the female's dietary requirements needed to trigger natural mating behaviours (Kubli, 2010).

On the day of the assay (day 11 of the culture cycle), males and females of all possible body size combinations were transferred without anesthesia into arenas using an aspirator, to avoid potentially confounding effects of CO_2 on behaviour (Barron, 2000), and filmed using high-definition video cameras (JVC Everio). Each treatment consisted of 25 replicates for a total of 300 pairs of flies. Filming occurred from 11:00h to 13:00h EST. Footage from each video camera was converted from .MTS to .AVI format using Aunsoft MTS Converter (http://www.aunsoft.com/mts-converter), and replayed using Windows Media Player 11 (Microsoft Corporation). For each pair of flies, the following pre- and post-copulatory variables were measured (in seconds): time to courtship initiation (TCI), length of courtship (LoC), length of copulation (LC), and incidence of successful courtship. TCI was defined as the point when the male began wing-vibration in an attempt to court the female (Bastock and Manning, 1955). LoC was defined as the difference in time between when copulation was initiated and the time when courtship

was initiated (TCI). LC was defined as the time from when copulation started (male mounts the female) and when copulation ended (male dismounts the female). These variables were specifically chosen because they reflect aspects of male and female pre- and post-copulatory behaviour that were expected to vary with body size (Partridge *et al.*, 1987a; Lefranc and Bundgaard, 2000). Relative body size comparisons were also considered for visualizing effects of body size on the aforementioned mating variables.

2.2.1 Statistical analysis

Initially none of the measurement variables (TCI, LoC, and LC) fulfilled the parametric assumptions of normality and homogeneity of variance. TCI and LoC were log-transformed to meet parametric assumptions. As I was unable to transform LC to meet assumptions of normality/homogeneity, I performed a rank transformation as per Conover and Iman (1981) prior to analysis. For examining absolute body size, models contained male treatment, female treatment, and their interactions as fixed effects. Two-way ANOVA compared the mean time until the relevant mating-related event occurred (i.e. courtship initiated or copulation initiated) of males and females belonging to different absolute body sizes, while one-way ANOVA compared different relative body size classes. Multiple *post hoc* comparisons between male and female groups were evaluated with Tukey's HSD test. To examine the frequencies of failures to either initiate courtship, or to successfully copulate during the observation period, data was analyzed using Generalized Linear Models (GLMs), where I used a logit link function and binomial error distributions (as is appropriate for dichotomous data). Models either consisted of absolute male and female body sizes (and their interaction), or relative body size scores. To better understand the relationship between each measurement variable,

Spearman's rank correlations were calculated. SPSS statistics (v20.0, IBM) was used to perform all statistical analyses, save those involving GLMs that were run using JMP (v. 8.0.1, SAS Institute, Carey, NC).

2.3 Results

Of the 300 pairs of flies that were observed, copulation did not occur in 146 cases. Data on LoC and LC from the 154 mating pairs were used in subsequent analyses. The likelihood of courtship initiation did not depend on the absolute size of males, females, or their interaction (whole-model GLM, $\chi^2 = 17.8253$, df = 11, $p = 0.0857$), nor on their relative body size (whole-model GLM, $\chi^2 = 0.291$, df = 2, $p = 0.865$). Likelihood of copulation did not depend on the absolute size of males, females, or their interaction (whole-model GLM, $\chi^2 = 11.69$, df = 11, $p = 0.38$), nor on their relative body size (whole-model GLM, $\chi^2 = 0.627$, df = 2, $p = 0.731$).

For those pairs of flies that did copulate, measurement of TCI using two-way ANOVA revealed no significant effects of either absolute male body size, female body size, or their interaction ($F_{6,11} = 1.262$, $p = 0.279$) (Table 3). When data were analyzed according to the relative body size of the flies, there was evidence of differences between groups ($F_{1,2} = 3.183$, $p = 0.044$). Specifically, when the female was larger than the male, the time to courtship initiation was, on average, 64.4s shorter than when females were of equal size to males (Tukey HSD; $p = 0.039$) (Figure 3). For those pairs of flies where there was courtship, but no copulation, measurement of TCI revealed no significant effects of either absolute male body size, female body size, or their interaction (ANOVA, $F_{6,11} = 1.106$, $p = 0.365$), or relative body size (ANOVA, $F_{1,2} = 1.080$, $p = 0.343$).

For those pairs of flies where a length of courtship was recorded, one-way ANOVA revealed no significant differences based on relative body size ($F_{1,2} = 0.636$, $p = 0.531$). However, when data was analyzed according to absolute body size using two-way ANOVA, there was some evidence of differences within female treatments ($F_{3,11} = 2.275$, $p = 0.083$). Specifically, extra-large females receieved 471.5s more courtship than small females (Tukey HSD; $p = 0.041$) (Figure 4).

ANOVA revealed no significant effects of either relative body size (ANOVA, $F_{2,151} = 0.206$, $p = 0.814$), or of absolute male body size, female body size, or their interaction on length of copulation (ANOVA, $F_{11,151} = 0.543$, $p = 0.871$) (Table 1 and 2).

Spearman's rank correlations revealed a significant positive correlation between LoC and LC ($N = 154$, $r_s = 0.194$, $p = 0.016$).

2.4 Discussion

That empirical evidence be replicable is the foundation of the scientific method and lends support to theories that predict a particular outcome (Kelly, 2006). While exact replication has its place in re-affirming foundational studies in a given field, exact replication is often expensive, time consuming, tedious, and provides no novel insight into the field (Kelly, 2006). Conversely, not conducting (and by extension not publishing) replicate studies greatly hinders efforts to derive general understandings of evolutionary phenomena; single studies, however significant, are not sufficient for the experimental demonstration of any natural phenomenon (Fisher, 1974). As the role of male and female body size on mating dynamics is potentially important to understanding sexual selection, I were motivated to use the model species *D. melanogaster* to

partially replicate Turiegano *et al.*'s (2012) study on the effect of male and female body size using a different base stock of flies to investigate similar measures of mating behavior: continuous pre and post-copulatory events that were known (or suspected) to individually vary with male and female body size. Using a different base stock of flies provides a different genetic background, selective history, and range of phenotypes and thus contributes to a more comprehensive understanding of how *D. melanogaster* (as a species) respond to variation in body size. Prior to my and Turiegano *et al*'s (2012) study, the concurrent effect of both sexes' body size variation on mating behaviours had not been investigated comprehensively. In my study, I confirm that varying male and female body size has significant effects on behavioural events that occur prior to copulation.

First, the likelihood of courtship initiation did not depend on either the absolute or relative body size of males or females. This was an unexpected result; short-term measures of male mating success in insects are often associated with size, as larger males may have more energy or may be better able to locate females and track them during courtship (Partridge and Farquhar, 1983). Additionally, large-bodied female *D. melanogaster* are typically observed to be courted more quickly than smaller females, as fecundity is directly related to body size (Alpatov, 1932; Byrne and Rice, 2006; Long *et al.*, 2009; Edward and Chapman, 2012). However, given that my chambers were of a small size (where flight was severely restricted) and that each chamber was well lit, there may not have been large costs/challenges of locating each other.

Time to courtship initiation was found to occur sooner in those cases where females were relatively larger than males, compared to when females were of similar size to males (in those cases where copulation occurred within the assay's time frame). While

this result was consistent with past work that quantified courtship initiation with respect to male body size (Byrne and Rice, 2006), I did not find a significant difference in courtship initiation times between cases when females were larger than males, and when females were smaller than males. Partridge *et al.* (1987a) suggested that males that are larger than females may have mating advantages due to higher levels of courtship behavior (i.e. more courtship attempts). In addition, it may be that smaller females are exerting preference for larger male body size, as females seem to prefer males that are harmful to them and may show higher net fitness through production of more fecund daughters and "sexy sons" (Pitnick and Garcia-Gonzalez, 2002). Turiegano *et al.* (2012) also found that a large size difference between males and females increased time to courtship initiation, consistent with my findings.

Furthermore, I found that likelihood of copulation was not affected by either male or female absolute or relative body size. The absence of an effect of body size may be due to the use of virgin flies in my experiment, which may have resulted in less discriminatory mate choice than would be observed had I used non-virgins. In addition, my small sample size (N = 154) may contribute to the unexpected result that copulation likelihood was not affected by either sex's body size. In contrast to my study, Turiegano *et al.* (2012) observed that female size significantly affected the likelihood of mating. As Turiegano *et al.* (2012) point out, larger females tend to keep moving for longer periods of time during courtship compared to smaller females, which would explain a female size effect; however, given that there was no effect of male body size, and that large male body size typically confers an advantage to the extent that females exert mate choice, preference for large size is considered to be the general outcome (Darwin, 1871;

Andersson, 1994; Pitnick and Garcia-Gonzalez, 2002). That this was not observed in Turiegano *et al.*'s (2012) study may indicate a lack of significant body size variation, as flies randomly sampled from a normal distribution of body sizes are less likely to include individuals from the tail ends of the distribution. Furthermore, Turiegano *et al.*'s (2012) use of an isofemale line of flies reduces the amount of genetic variation present in their study and may be more appropriately used for examining the genetic variability present in natural populations and investigating genotype-environment interactions (David *et al.,* 2003).

Analysis of length of courtship revealed that "extra-large" females received longer courtship than did small females. Little work has been done that has quantified the effect of body size on courtship length; past studies have primarily examined the effects of female age (e.g. Connolly and Cook, 1973), whether females were virgin or non-virgin (Bastock and Manning, 1955; Friberg, 2006), or the effects of specific mutations (e.g. Roche *et al.,* 1998). Given that previous studies have reported that larger females are more preferred by males (Pitnick and Garcia-Gonzalez, 2002; Byrne and Rice, 2006; Long *et al*., 2009), it is conceivable that larger females (that had longer lengths of courtship) are exercising a greater degree of choosiness than small females and require more male effort before they copulate (Cotton *et al.*, 2006), though this choosiness would likely be modulated by male body size. That I did not find an interaction between male and female size was therefore surprising. As mentioned previously, larger males tend to be more active than smaller males and can move faster (Partridge *et al.* 1987a); this potentially makes them better able to track females when they prior to courtship, which likely contributes to larger males having shorter courting times than smaller males

(Fulker, 1966). The number of individuals may have contributed to the lack of interaction, as 154 pairs never copulated (and therefore did not contribute a LoC measurement). Contrary to my findings, Turiegano *et al.* (2012) reported no effect of male or female body size on length of courtship.

Length of copulation analyses revealed no effect of either absolute or relative male or female body size. This was surprising, given the number of studies reporting variation in copulation length based on body size (Pitnick, 1991; Lefranc and Bundgaard, 2000; Pitnick and Garcia-Gonzalez, 2002). However, both Lefranc and Bundgaard (2000) and Pitnick and Garcia-Conzalez (2002) used Oregon-R in their experiments, a highly inbred stock that could possess extremely limited genetic variation. Pitnick (1991) used two stocks that had only adapted to the lab for 3-6 generations at room temperature and therefore may have been more susceptible to physiological stressors than longer-established stocks (Hoffman *et al.*, 2001). In addition, the above studies generated body size variation by manipulating larval competition conditions. Larval crowding affects nearly all components of fitness, including body size and female fecundity (Alpatov, 1932; Ashburner *et al*., 2005). It is therefore possible that larval crowding affects copulation duration, as it can lengthen the developmental period of both sexes, affecting both reproductive and somatic systems in adult *D. melanogaster* (Ribó *et al*., 1989). In contrast, my experimental flies' body size variation was generated in a way that did not manipulate larval competition conditions, and my experimental flies used were an outbred population that had been lab-adapted for >900 generations (see 2.2 Materials and Methods). These methodological differences may have influenced my results when contrasted with the studies previously mentioned.

Finally, Spearman's rank correlations revealed a significant positive correlation between LoC and LC. Past studies have reported significant interactions between female choosiness and female body size (Friberg and Arnqvist, 2003; Byrne and Rice, 2006), and while my study did not find evidence of copulation duration being affected by body size, it is conceivable that choosier females (i.e. larger females) copulate longer when able to freely exercise mate choice (as opposed to scramble scenarios/male-male competition). Given the amount of methodological variation present in past studies that have examined length of copulation and the lack of studies examining length of courtship in *D. melanogaster*, that I only found a correlative relationship between these two factors invites further study into the complex relationship between body size and mating behaviour.

My assay provides additional evidence that *D. melanogaster* males and females both evaluate potential mates on the basis of both absolute and relative body size differences, and that the body size of both sexes directly affect time to courtship initiation, length of courtship, and length of copulation. While body size as a predictor of mate choice choosiness has typically been congruent with predictions made by sexual selection theory (Andersson, 1994), methodological differences between studies have made interpreting the contribution of each sex's body size variation difficult to integrate into a more general framework. Furthermore, while female mate choice is beginning to receive more attention in relation to between-sex body size variation, factors influencing how female mate choice preference might evolve in a population are currently unknown. Together, my results invite further study of between-sex body size variation in order to

comprehensively model the interactions between male and female *D. melanogaster* mating behaviours.

2.5 Figures and Tables

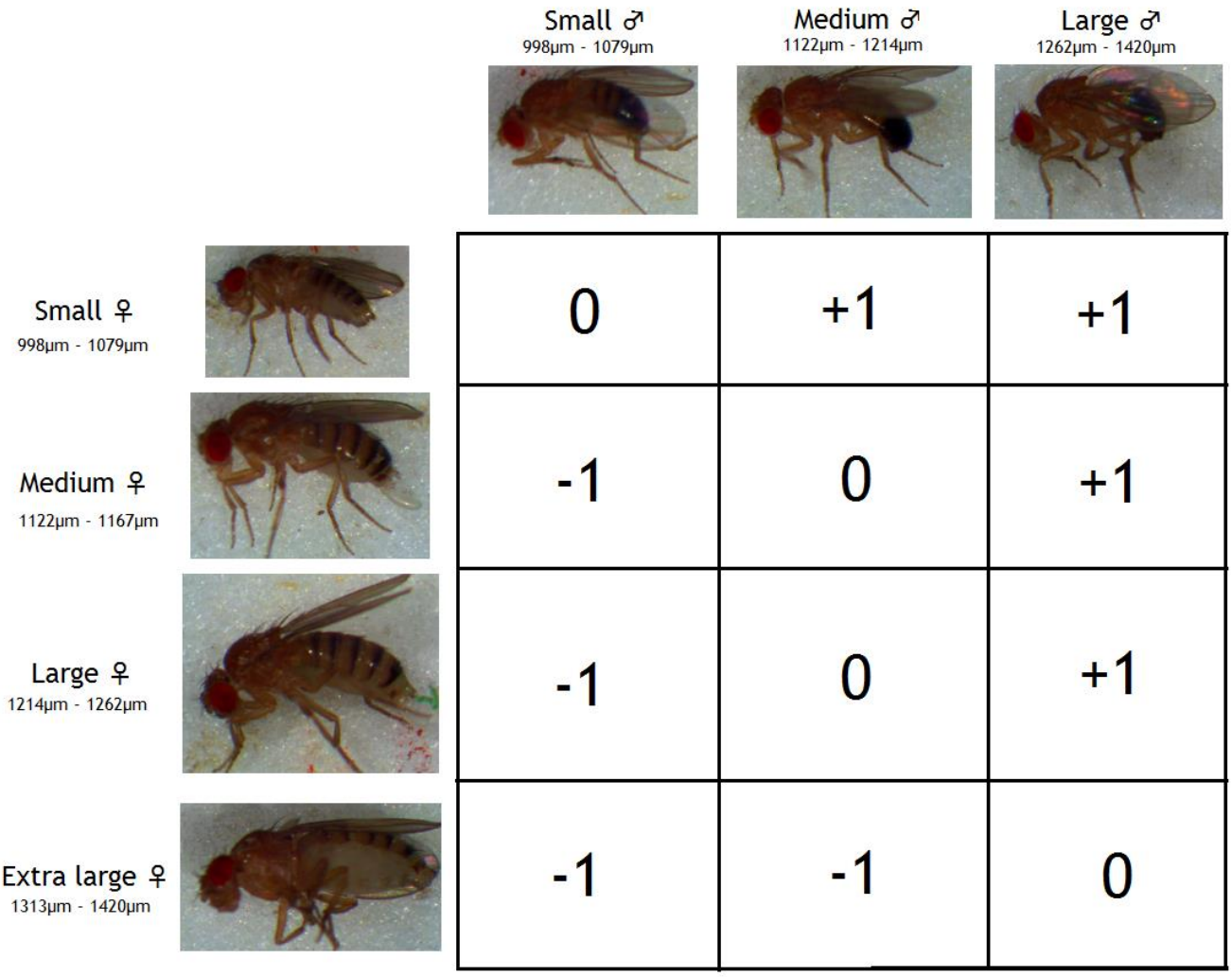

Figure 1. Images of representative *D. melanogaster* males and females of various size classes used in experiments, and table illustrating the relative body size coding scheme used. For relative codings, positive values indicate pairs where females are smaller than males. Negative values indicate pairs where females are larger than males. Zero values indicate pairs where females and males are of similar size.

Figure 2. A mating arena used to observe pre and post-copulatory mating variables between male and female *D. melanogaster*. The square around the arena is a transparent plastic covering that allowed for direct observation and also prevented the flies from escaping (secured to the arena by a clip).

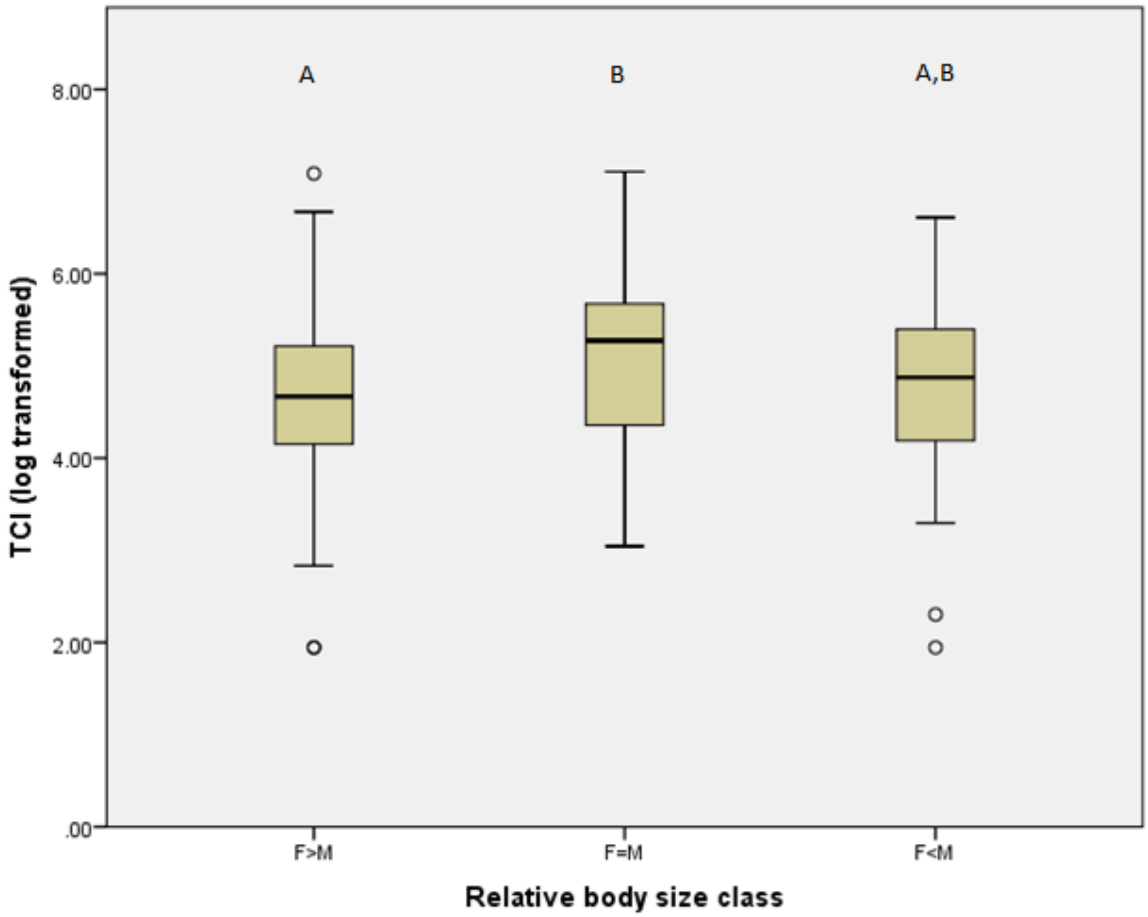

Figure 3. Log-transformed TCIs for each relative body size class. F>M are cases when females are larger than males, F=M are cases when females and males are of equal size, and F<M are cases when females are smaller than males. Males began courting females significantly sooner when females were relatively larger than males, and significantly later when females were approximately equal in body size to males. Top and bottom boxplot whiskers represent 1.5 times the interquartile range for each respective relative body size class. Open circles represent outliers. Different letters above each boxplot represent body size pairings that showed significant differences.

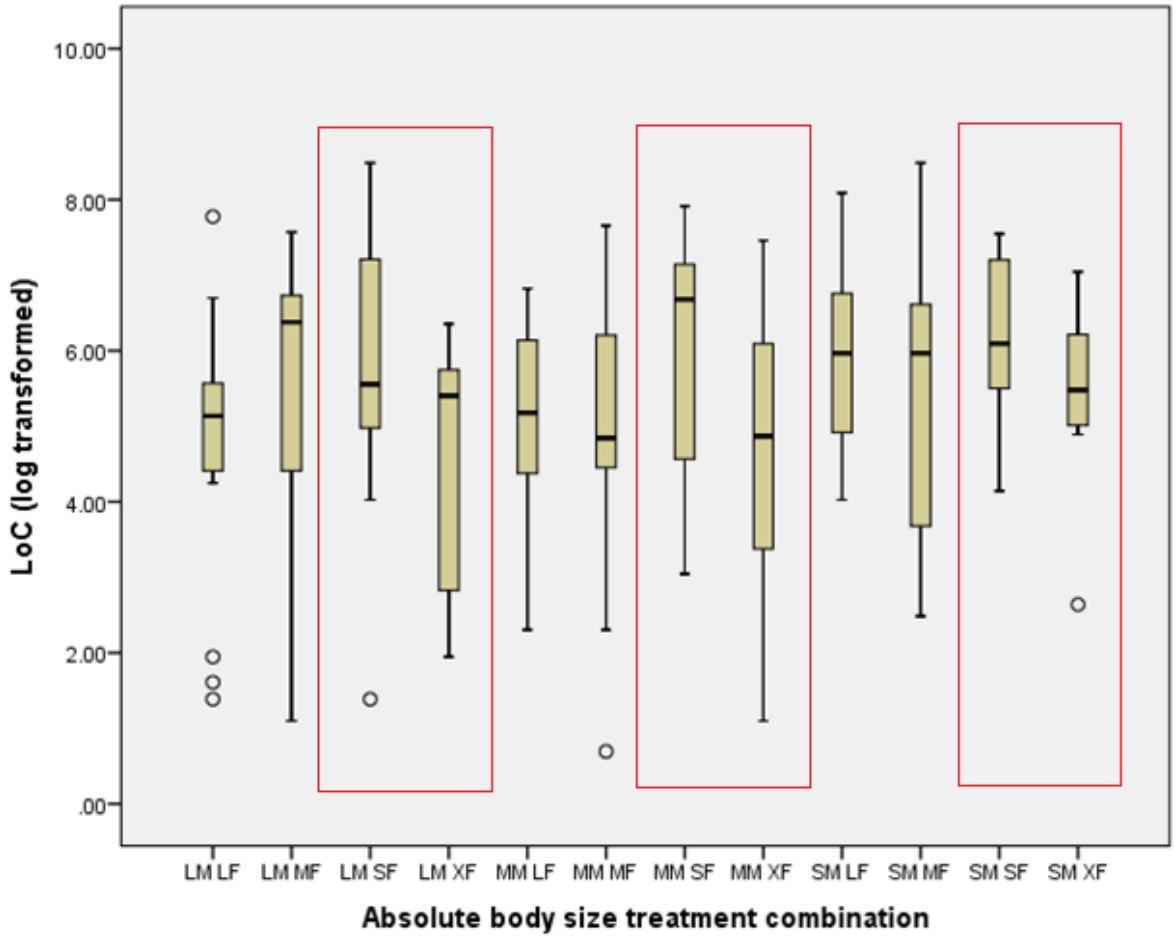

Figure 4. Log transformed LoCs for each absolute body size treatment combination. Male size, followed by female size, on the x-axis denotes each absolute body size pairing (e.g. SM XF = small male, extra-large female). Small females were courted significantly longer than extra-large females, regardless of male body size (highlighted in red). Top and bottom box plot whiskers represent 1.5 times the interquartile range for each respective absolute body size class. Open circles represent outliers.

Abs male size		TCI	LoC	LC	N
	Abs female size				
SM	SF	337.6 ± 66.7	1935.2 ± 455.8	1239.1 ± 77.8	15
	MF	446.8 ± 66.2	2095.4 ± 457.5	1801.7 ± 370.7	18
	LF	619.142 ± 129.8	1313.4 ± 383.3	1208.3 ± 51.8	12
	XF	696.572 ± 139.1	896.9 ± 185.3	1221.1 ± 56.3	9
MM	SF	501.9 ± 66.1	2333.3 ± 514.6	1323.4 ± 87.8	11
	MF	443.8 ± 73.2	2068.5 ± 499.0	1615.9 ± 262.1	15
	LF	585.7 ± 133.5	1670.7 ± 370.9	1213.8 ± 55.9	11
	XF	654.4 ± 151.3	949.0 ± 184.8	1220.0 ± 55.9	11
LM	SF	307.5 ± 57.1	2555.1 ± 504.9	1210.6 ± 68.9	14
	MF	520.2 ± 77.0	2358.9 ± 531.4	2413.9 ± 284.1	11
	LF	554.4 ± 115.2	1430.4 ± 312.7	1161.5 ± 47.1	15
	XF	437.1 ± 101.2	840.1 ± 173.8	1192.2 ± 50.6	12

Table 1. Mean ± standard errors (in seconds) for time to courtship initiation (TCI) for those that ultimately mated, length of courtship (LoC), and length of copulation (LC) for each absolute (abs) body size combination.

Relative body size	TCI	LoC	LC	N
Female > Male (-1)	325.4 ± 49.0	562.8 ± 108.4	991.9 ± 33.5	60
Female = Male (0)	436.5 ± 81.4	449.4 ± 73.8	971.4 ± 39.5	53
Female < Male (1)	242.8 ± 33.4	705.7 ± 164.6	997.3 ± 77.4	41

Table 2. Mean ± standard errors (in seconds) for time to courtship initiation (TCI) for those that ultimately mated, length of courtship (LoC), and length of copulation (LC) for each relative body size combination.

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3.0 Effects of experimental evolution on female mate preference for male body size in *Drosophila melanogaster*

3.0.1 Abstract

Past studies have suggested that direct selection has the potential to cause substantial evolutionary change in female mate choice. However, few studies have directly tested whether female preference variation has a genetic component. Here, this question has been addressed using experimental evolution in the fruit fly, *Drosophila melanogaster*, in which female mating preferences were selected upon for an attractive male trait (body size). I found that female preference responded to direct selection over multiple generations, with female flies associating significantly less with males of the body size class with which they were selected against. Furthermore, I found that mean body size decreased in some treatments, suggesting that my selection on female preference had a genetic correlation with its corresponding male trait. This study revealed that female preference may have a genetic component that is capable of being strongly shaped by sexual selection.

3.1 Introduction

Rather than being uniform, in many species individual females vary in their preferences for male traits. Some of this preference variation is environmental in origin; individuals can vary widely in condition, creating significant differences in their ability to express mate preference(s) (Jennions and Petrie, 1997). Local environmental conditions can further influence the amount of time and energy required for locating and sampling potential mates, increase predation risk, and influence available territory or resource quality (Jennions and Petrie, 1997), all of which may affect female preferences. However, preference variation may also have a genetic component: if preference functions and mate sampling behaviours have heritable components, they may show an evolutionary response to selection (Widemo and Sæther, 1999). Genetic variation in female preferences can influence both the strength and the shape of selection acting on male display traits, ultimately having profound implications for the rate and direction of evolutionary change (Jennions and Petrie, 1997). Mate choice based on a particular trait will cause both the genes that influence both that trait and its corresponding female preference to reside in the same offspring; selection for a preferred trait may also indirectly select on preference for that trait (Bateson, 1983). Ultimately it is the genetic variation which is important to evolutionary biology and, as such, understanding the type and amount of variation is of great importance.

Most evidence for genetic variation in female preferences has come from comparative studies of species where male sexual traits vary geographically. Ritchie (1991) demonstrated with bushcrickets (*Ephippiger ephippiger*) that there was wide variation in male song characteristics between populations. Subsequent investigations of

female mate choice using synthetic songs generated in a laboratory environment showed that females strongly preferred male songs from their native population, with the genetic component later demonstrated directly in crosses between populations (Ritchie, 1992). Similarly, geographic variation of male sexual traits has been observed in guppies (*Poecilia reticulata)*. Guppies exhibit sexual dimorphism, with males having bright orange spots on their body which attract females (Houde, 1988). However, as the same colour patterns and brightness increases their visibility to predators, sexual selection and predation enforce a balance between colour pattern/brightness parameters. Endler and Houde (1995) collected guppies from 11 different locations, each varying in predation intensity, male color pattern, body shape, size, and overall colour and brightness, to test if geographical variation in female preference was related to the observed geographical variation in male traits. Females were found, on average, to be more attracted to males from their own population than to males from other populations, with populations varying in criteria used in female choice. While some studies have suggested that there is little to no genetic variation in female preferences (Paterson, 1985; Boake, 1989), more and more studies are finding evidence for phenotypic and additive genetic variation in several components of female mating preferences, having been observed in house finches (*Carpodacus mexicanus*) (Hill, 1991), ladybirds (*Adalia bipunctata*) (Majerus *et al*., 1982, 1986) and in several species of fruit fly (Klappert *et al*., 2007; Sharma *et al*., 2010; Bailey *et al*., 2011).

Despite a number of past studies having examined maintenance of female preference variation between populations (Bakker and Pomiankowski, 1995; Houde and Hankes, 1997), variation in mating preferences *within* populations has received less

attention. One reason for this lack of information may be due to limitations associated with experimental design. Traditional female mate choice assays typically use population-level preference tests (whereby many females from a population are tested once with a set of stimuli), two-stimulus tests (often chosen from extremes of a phenotypic distribution), or simultaneous-stimulus tests. While their designs have the advantage of being easy to conduct and whose results are relatively easy to analyze, they often make it difficult to distinguish variation between individuals (with respect to preferences) due to several factors (Wagner, 1998). Such experiments may mask potential variation in preferences between females; if some females prefer "higher" trait values and some females prefer "lower" trait values, tests for female preference in a given population may falsely indicate that females do not exhibit mate choice preference based on that trait (Wagner, 1998). Furthermore, traditional studies may only allow for directional preferences to be analyzed (i.e. when a female's ideal mate is significantly different in phenotype from their own (Kirkpatrick, 1987)). Instead, a more efficient and appropriate method to test whether genetic variation exists within populations for female preferences is to use experimental evolution.

Experimental evolution is an approach that allows investigation of how heritable traits respond directly (or indirectly) to selection over multiple generations (Garland and Rose, 2009). By studying replicate populations over time under standardized and replicable conditions, co-evolution of a male trait and female preference for that trait may be directly tested. Experimental evolution has been widely used in microorganisms such as *Escherichia coli* (Elena and Lenski, 2003) and *Saccharomyces cerevisiae* (Zeyl, 2006*;* Parts *et al*., 2011), as well as in multicellular organisms such as *Poecilia reticulata*

(Houde, 1994; Hall *et al*., 2004) and in a variety of *Drosophila* species (Hoffmann *et al*., 2003; Turner *et al*., 2011; Zhou *et al*., 2011) to study within-population trait evolution. Though male ornamentation has been shown to have substantial variation and heritability (Andersson, 1994), few studies have used experimental evolution to examine whether variation in female preferences exist. One of the largest studies done to investigate this was done by Houde (1994), who used artificial selection on male guppies (*P. reticulata*) over 3 generations to determine if a genetic correlation existed between a male display trait (orange colouration) and female preference for that trait. If a correlation existed, artificially selecting for increased or decreased amounts of orange colour in males should result in a corresponding shift in female mating preference. Houde (1994) found that females from the treatment in which males were selected for increased colouration showed stronger preference for orange than females from the treatment selecting for decreased colouration after only two generations. While these results may suggest that female preference in each treatment evolved as a correlated response to the artificial selection on male colour, how female preferences evolve over longer periods of time remains unclear, inviting further investigation as to how sexual selection may be influencing them. Conversely, Hall *et al*. (2004) similarly used artificial selection on guppies (*P. reticulata*), though they directly selected on female preference for male colouration and on male colouration itself over 3 generations. However, unlike Houde (1994), Hall *et al*'s (2004) artificial selection failed to produce a response. Several reasons were suggested for the lack of response, including low heritability of the male attractive trait and female mate choice, large environmental variances via measurement error, and treatments having different local fitness optima (Hall *et al*., 2004). While this

finding posed the important question of if female preference has a genetic component in guppies, its results demonstrated that the responses of female preferences and male traits to selection may be constrained by a number of different factors.

Fruit flies (*Drosophila melanogaster*) have been used to investigate questions in the field of population genetics since at least 1952 (Robertson and Reeve, 1952). While numerous studies have experimentally evolved body size (e.g. Huey *et al*., 1991; Partridge and Fowler, 1993; Partridge *et al*., 1999; Turner *et al*., 2011), a trait with considerable genetic variation, to the best of my knowledge, no past experimental evolution studies using *D. melanogaster* have examined female preference evolution for male body size. As female *D. melanogaster* can actively reject "unwanted" male copulation attempts (Spieth, 1952; Dickson, 2008) and that females have been shown to display preference variation in a variety of traits (Heisler, 1984; Greenacre *et al*., 1993; Bailey *et al*., 2010; but see Long *et al*., 2009) which may be correlated with variation in male body size, there is conceivably strong potential for sexual selection to shape female mate choice evolution for male body size.

In *D. melanogaster*, mate preference based on male body size has been shown to be an important factor in several aspects of pre- and post-copulatory behaviour. Larger *D. melanogaster* males have shorter times to copulation initiation (Partridge *et al.,* 1987b), are able to stimulate short-term female fecundity more than smaller males (Friberg and Arnqvist, 2003, but see Pitnick, 1991), and transfer more sperm during copulation than smaller males (Lupold *et al.,* 2011). Females that mate with larger males, however, suffer greater negative direct fitness consequences compared to those mated to smaller males due to a combination of physical harm incurred during courtship/copulation and the toxic

side-effects of the accessory proteins present in the seminal fluid (Pitnick, 1991; Friberg and Arnqvist, 2003; Long *et al.,* 2010). That we have observed that larger males copulate more quickly with females than smaller males may suggest females prefer this trait (Fulker, 1966). However, the advantages that larger males had in obtaining shorter times to copulation may have been primarily due to their increased physical abilities in coupling with females, especially with smaller females having a decreased ability to resist mating (Partridge *et al.,* 1987a; Turiegano *et al.,* 2012). As past studies have typically varied body size of members of a single sex (Pitnick, 1991), and have given relatively little attention to female preference variation, the co-evolutionary dynamics of these two traits remains unclear.

Here, I use an experimental evolution approach to examine whether females possess genetic variation for mate preference with respect to male body size. This was done by imposing artificial selection in replicate populations of fruit flies on different body sizes using two independent *D. melanogaster* populations. I "penalized" females that mated with males of a particular body size treatment by discarding their offspring in every generation for 20 generations. I predicted that female preference would evolve in a way that causes them to avoid associating or mating with males of the size phenotype that carried this extra direct cost. To determine if change in female preference resulted in responses in body size (directly in males and indirectly in females), I quantified male and female body size variation present at each generation. Additionally, female preference behavioural assays were periodically conducted at regular intervals during the study. Together, these assays are designed to reveal whether or not genetic variation in female preference for body size is present.

3.2 Materials and Methods

3.2.1 Population Origins and Culture Conditions

The stocks used in this experiment were IV, IV-bwD, and LHm. The IV (*Ives*) stock is a large, outbred wild-type stock descended from a population of *D. melanogaster* collected in South Amherst, Massachusetts, USA in 1975 that has been maintained in laboratory for >900 generations (Rose, 1984). IV flies used were cultured at a density of 100 individuals/vial (as per their typical culture protocol). The IV-bwD stock was generated by repeatedly backcrossing a brown-eyed dominant mutation (bwD) into the IV population. IV-bwD flies were also cultured at a density of 100 individuals/vial. Finally, the LHm stock is a large, outbred wild-type population descended from 400 females collected in central California in 1991 and have been maintained in the laboratory for >500 generations (Rice *et al*., 2005). LHm flies were cultured at a density of 150-180 individuals/vial (as per their typical culture protocol).

The IV, IV-bwD, and LHm stocks were maintained on a standardized 14-day culture cycle with non-overlapping generations. All flies were housed in a humidity-controlled incubator on a 12 hour light/dark diurnal cycle at 25º C and kept on standard banana/molasses/corn syrup/killed-yeast media (Rose, 1984). Each generation begins on "day 0", where vials contain a standardized density of eggs. Flies began eclosing from their pupae starting on day 9 of the culture cycle. For both the IV and IV-bwD stocks, on day 14, stocks are propagated by lightly anesthetizing flies with CO_2 and mixing individuals from all vials of the same population, then re-distributing them into an equal number of "egg-laying" vials containing 10mL of standard medium. Several hours later, flies in each population are removed from "egg-laying" vials and the number of eggs in

each vial was culled to their standard densities. For LHm flies, individuals are transferred on day 12 to new vials for 48h before being transferred to egg laying vials, where the egg density is also standardized (refer to Rice *et al*., 2005 for full details).

3.2.2 Experimental Populations – Origin and Initial Generations

Both IV and LHm stocks were used to generate experimental evolution populations (henceforth IV-EE and LHm-EE populations). From each stock, six populations, each consisting of 7 vials of flies (at their traditional densities) were created. Simultaneously, four paired IV-bwD replicate populations consisting of 14 vials per population (at 100 eggs/vial) were derived from laboratory IV-bwD stocks and subsequently maintained and cultured in parallel with IV-EE and LHm-EE lines for the duration of the study.

In order to select on female preferences, I subjected populations of IV-EE and LHm-EE flies to selection pressure where mating with males of a certain body size phenotype was penalized. This was accomplished by first sorting IV-bwD males collected from each of the paired replicate populations into three different body size classes using a Gilson Company Inc. Performer III model SS-3 sieve shaker (as per Long *et al*., 2009). The sieve shaker mechanically separates flies using 11 successive sieve plates with a 5% difference between electroformed hole diameters in each plate (diameter of top-most sieve holes = 1420 µm, diameter of bottom-most sieve holes = 998 µm). Once the flies were placed in the top sieve of the column (under light CO_2 anesthesia), the sieve shaker was activated at a rate of 3600 vibrations min^{-1} for two consecutive two minute periods, facilitating the flies' downward movement. This approach was used in favor of the traditional larval crowding or varying larval food quality because of both the

ease with which hundreds of flies can be sorted simultaneously (Long *et al*., 2009) and also because the body size variation more accurately represents the phenotypes which typically exist within each source population. IV-bwD male size categories were chosen based on a body size distribution of 350 male IV flies quantified in a pilot study (Figure 5). Each treatment category was defined by a thoracic diameter range: "large" males had a thoracic diameter greater than 1167um, "medium" males were between 1122-1079um, and "small" males were smaller than 1038um. IV-bwD males from each size class were then assigned to an EE line treatment. The same IV-bwD population provided males for the same set of 3 EE populations throughout the course of the study (Figure 6). Females that mated with IV-bwD males (of a specific body size) were artificially penalized by discarding all brown-eyed offspring that were subsequently produced in the next generation (described below).

Starting 9 days after eggs had been initially laid in vials by the first generation of IV-EE and LHm-EE line flies, newly eclosed adult virgin males and females were collected 3 times daily, with each collection separated by 6-8 hours, to minimize potential mating among eclosing adults. Flies collected were separated by sex and placed into food vials at a maximum density of 75 individuals/vial. Collections continued until 350 males and 350 females were obtained from each EE population. On day 10, IV-bwD males were separately collected for each of the 4 replicated populations and sorted by size using the sieve sorter (described above). From each of the IV-bwD populations, four vials (each containing 75 males) were collected for each size class, for a total of 12 vials.

On day 11, males and females collected from each EE line were placed into population cages constructed from Ziploc "Twist 'n Loc" 946mL containers (Figure 7),

which contained medium with additional live yeast. IV-bwD males of the appropriate size class for the treatment were then simultaneously introduced into each population cage along with the wild-type males and females until each chamber contained 425 (75 brown-eyed + 350 wildtype) males and 350 females. Flies were left to interact in the mating cages for the next two days in the incubator. On day 14, medium in each chamber was replaced with 7 vials, each containing 10 mL of medium, with additional live yeast added. Eggs laid in these vials established the next generation of each EE line. After 6-8 hours, vials were removed from all chambers and trimmed to the density appropriate to each EE line's source stock. In all subsequent generations, wild-type male and female flies that eclosed from EE lines were collected starting on day 9, with any eclosing bwD offspring being discarded. Subsequent generations repeated the above procedure of sorting IV-bwD males from each paired replicate population into the three size classes, which acted as the source of artificial selection on female preference for each body size treatment.

3.2.3 Experimental Evolution – Effects on Body Size, Sex, and Eye Phenotype

Once the eggs needed to propagate the EE lines had been collected, two 35mm diameter petri-dishes containing grape juice agar (Sullivan *et al.,* 2000) with a small amount of live yeast paste were added to each cage and left overnight. On day 15, the petri-dishes were retrieved and from the eggs oviposited on their surface, sets of 100 or 150 eggs each (for IV and LHm-derived EE populations, respectively) were moved to vials containing 10mL of medium. For each EE population in each generation, two vials of eggs were created and allowed to develop in the incubator for 14 days. Polyvinyl tubing "extenders" were attached to each vial to facilitate collection of eclosed adults.

Flies that eclosed from these vials were then sorted by body size using the methods described above, and sex and eye colour phenotype in each size class was counted. This was done to determine if body size of wild-type flies in the EE lines were responding to the selection and if brown-eyed dominant offspring frequency was changing over time. This protocol was repeated for 20 generations.

3.2.4 Female Behavioural Assay

In order further quantify female mate preferences for male body size in each EE line over the course of the study without potential male-male intrasexual competition confounds, behavioural assays were conducted at generations 2, 6, 10, 15, and 20. For these assays, females were obtained from each of the IV-EE and LHm-EE lines, while males were obtained from the IV and LHm lab stock populations (respectively). From each IV and LHm lab stock, five separate populations (7 vials of eggs at their typical densities per population) were established. Males were used from IV and LHm lab stocks rather than the EE lines, as doing so removes the potential confounds of co-evolutionary change that may have occurred in the males as a result of the artificial selection.

Female flies used in assays were collected by introducing a set of 7 vials for ~2 hours into the population cages after eggs from the culturing vials and grape juice plates had been collected. This was done on two consecutive days. Subsequent collections of LHm and IV males from source stocks were timed so that they would be of the same age as EE females used in the assay. This allowed each assay to be split across 2 days, with 16 replicate females assayed on each day for each EE-derived population for a total of 32 replications/population/trial. From each set of vials, wild-type females were collected as virgins within 6-8 h of their eclosion starting 9 days after oviposition. Virgin females

were kept in vials containing food media at a maximum density of 16 females vial^{-1} and housed in a temperature-humidity controlled incubator. Collections continued until a minimum of 48 females were obtained from each set of EE lines.

On the day that preceded each assay day (which corresponded to day 12 for the EE-assay vial females - the day that they typically first encounter males in their EE culture protocol), IV and LHm males from vials derived from the laboratory stocks were collected. Males collected from both stocks were separated by size into three distinct size classes using the sieve shaker (described above). Males were kept in vials containing food medium at a maximum density of 75 males per vial and housed in a temperature-humidity controlled incubator. A minimum of 110 males from each laboratory-derived stock were collected for each day of the assay.

EE-IV and EE-LHm females were housed in chambers permitting close proximity, but not direct contact, to male flies in order to quantify any differences in behaviour resulting from selection (Figure 8). Each chamber contained an open area in which a female could move freely and interact, and four plastic "sub-chambers". Three of the sub-chambers each contained media and a single male of one of the three body size classes (described above), while the fourth contained only food medium to account for the event of "no male choice" by a female. Each sub-chamber was covered by a 125 micron polyethylene mesh glued to its opening, which allowed for females to sample auditory, visual, and chemical cues of the males in each sub-chamber without physically interacting with them (or being subject to male-male competition).

On the afternoon prior to the assay, males were placed into the female mate preference sub-chambers using light anesthesia and allowed to recover overnight in the

incubator, as CO_2 exposure can alter activity levels and fertility (de Crespingy and Wedell, 2008). The next day, females were placed into their preference chambers using light CO_2 anesthetic (< 30s exposure). Chambers were then mounted vertically on a corkboard. All chambers were sequentially rotated 90 degrees relative to each other when mounted on the cork board. While chamber orientation may potentially be an issue, standardizing chamber orientation over each generation (when a behavioural assay was conducted) controlled for any potential gravitaxic effects that may influence behaviour. Female preference chambers were videotaped using JVC high-definition Everio cameras using the UXP quality setting and 1 frame per second time-lapse setting for six hours (yielding ~21000-21600 frames/assay). To account for mechanical disturbances in recording equipment during setup and to ensure females were fully recovered from the light CO_2 anesthesia, the first 500 frames of each video were not analyzed.

In total, 32 total replications per EE line per generation were filmed. Video footage was subsequently converted to an HD format using Aunsoft MTS/M2TS Converter (version 1.3.6, Aunsoft, 2008) for scoring with VideoFly motion-tracking software. VideoFly (Kuo *et al*., 2012) was written in C and C# and was generously provided by Dr. Scott Pletcher (University of Michigan). For each female chamber, 4 regions of interest were defined (corresponding to the 4 sub-chambers, see Figure 8). The software was then tasked with identifying the location of the target female in each frame of each video. The total residence of each female in each region of interest was tallied.

3.2.5 Statistical Analyses

To determine if the frequency of brown-eyed progeny was changing over the course of the experiment, the fraction of offspring possessing wild-type eyes in each

population, in each generation, was calculated. A binomial test was then conducted for each population in which the frequency of wild-type eyed progeny in each generation was compared to the fraction at generation 0 (a control generation of IV or LHm flies wherein no artificial selection pressure was applied). In this analysis, the null probability being compared was 0.5. For each sex in each population, to determine if body size was increasing or decreasing in each treatment over time, one-way ANOVA followed by Dunnett's tests were conducted to compare the mean body size of generation 0 flies (control group) to those of flies in every subsequent generation. To determine if mean body size was changing over the course of the experiment, the number of times the mean size of flies in a treatment were smaller than the mean size of flies in generation 0 was calculated. A binomial test was then conducted for each sex in each treatment, where the mean body size for each generation was compared to the mean body size for that treatment at generation 0. In this analysis, the null probability being compared was 0.5. To determine if male and female body size varied significantly within each treatment over the course of the study, linear models were constructed. Finally, to determine how female behaviour responded to artificial selection over multiple generations, I examined the number of times sub-chambers containing a wild-type male of a particular body size were visited by females from each treatment. Data generated using VideoFly motion tracking software were analyzed by constructing Generalized Linear Models (GLMs) that used a logit link function and binomial error distributions (as is appropriate for dichotomous data), where the number of sub-chamber residence counts is the dependent variable, and the total number of sub-chamber residence counts (total sub-chamber associations) is the binomial denominator. Each model used fly stock (IV or LHm) and

the treatment generation of which the assay was conducted on as non-nested model effects. Contrasts were performed between body size treatments if the whole-model effect was found to be significant. For the small treatment, "small" body size class was compared against "medium" and "large" together. For the medium treatment, "medium" body size class was compared against "large" because past studies have found that females tend to prefer large-bodied males (Partridge *et al*., 1987b; Pitnick, 1991; Turiegano *et al*., 2012), despite the observation that females who mate with larger males have lower lifetime fitness (Pitnick and Garcia-Gonzalez, 2002). Finally, for the large treatment, "large" was compared against "medium" and "small" together. JMP (v. 10.0.0, SAS Institute, Carey, NC) was used to perform all statistical analyses, save boxplots which were generated using R (v3.0.2, R Core Development Team 2013).

3.3 Results

Binomial exact tests performed on EE-IV treatments revealed that over the course of the experiment the IV-M1 treatment vials possessed relatively more bwD flies than was expected by chance alone ($p = 0.0128$), while the IV-S1 and IV-S2 treatments possessed relatively fewer bwD flies than was expected by chance alone ($p = 0.0018$ and $p = 0.0026$, respectively). Only two EE-LHm treatments were found to have fewer bwD flies than was expected by chance in both LHm-S1 and LHm-S2 ($p = 0.0118$ and $p = 0.0118$, respectively). All other binomial exact tests performed on EE-IV and EE-LHm treatments were non-significant (all $p > 0.1$).

Dunnett's tests performed on mean body size calculated each generation (using Generation 0 as the control group) found significant changes within each EE population. In EE-IV and EE-LHm large treatments, male and female flies both significantly

decreased in mean body size with respect to generation 0 (Figure 9-12). In EE-IV medium treatments, female flies showed significantly decreased mean body size (Figures 14a and 14b). IV-M2 males were also found to show a significant decrease in mean body size at various generations and a general trend of decreasing body size (Figure 13b); however IV-M1 males showed a significant increase in mean body size in earlier generations (generations 3 and 5; Figure 13a), with only generation 12 showing a significant decrease in mean body size. Similarly, EE-LHm medium treatment male and female flies both significantly decreased in mean body size when compared to generation 0 (Figures 15 and 16). For EE-IV small treatments in both IV-S1 and IV-S2 treatments, males showed a significant decrease in mean body size in several generations (Figures 17a and 17b, respectively). Mean female body size significantly increased in earlier generations (generation 2 & 3 for IV-S1, generation 3 & 5 for IV-S2), mean female body size significantly decreased in both IV-S1 and IV-S2 treatments in several subsequent generations (Figures 18a and 18b, respectively). Finally, in EE-LHm small treatments, male flies from LHm-S1 showed significantly increased mean body size in several generations (Figure 19a); however, while LHm-S2 males showed a significant increase in mean body size in generations 2, 5, and 7, mean male body size significantly decreased in generations 6, 11, 13, and 16 (Figure 19b). Females from both LHm-S1 and LHm-S2 treatments showed a significant decrease in mean body size at several different generations (Figures 20a and 20b, respectively), with only LHm-S2 females showing a significant increase in mean body size at generation 7.

Binomial exact tests performed on mean body size for females revealed significant decreases when compared to generation 0 across all treatments (all $p < 0.05$)

with the exception of the IV-S1 and IV-S2 treatments ($p = 0.2632$ and $p = 0.8238$, respectively). For males, significant decreases in mean body size when compared to generation 0 were also found across all treatments, with the exceptions of IV-S1 ($p = 0.2632$), IV-S2 ($p = 0.5034$), IV-M2 ($p = 0.2632$), LHm-S1 ($p = 0.5034$), and LHm-S2 ($p = 1.0$) treatments.

Linear models fit to body size variation in each treatment over 20 generations revealed no significant variation for males (Table 3). However, females were found to have significant body size variation in the IV-M1 ($t = 2.498$, df = 19, $p = 0.022$) and LHm-M2 ($t = 2.138$, df = 18, $p = 0.046$) treatments (Table 4).

VideoFly data revealed for small treatments (Figures 21-24) that EE-LHm females showed significant differences in sub-chamber associations in assays conducted during generation 2 (whole-model GLM, $\chi^2 = 6.832$, df = 2, $p = 0.033$) and generation 15 (whole-model GLM, $\chi^2 = 10.675$, df = 2, $p = 0.005$) (Figure 21 and 22). EE-LHm generation 6 was marginally non-significant (whole-model GLM, $\chi^2 = 5.384$, df = 2, $p = 0.067$). Contrasts revealed marginally significant differences between individual body size class comparisons (generation 2, $p = 0.056$; generation 6, $p = 0.077$; generation 15, $p = 0.190$); females tended to avoid small-bodied males and associate with medium-bodied and large-bodied males. No EE-IV small treatment showed significant differences in sub-chamber associations at any generation (whole-model GLMs, all $p > 0.1$). For medium treatments (Figures 25-28), EE-IV females showed significant differences in sub-chamber associations in generation 6 (whole-model GLM, $\chi^2 = 10.303$, df = 2, $p = 0.006$), generation 10 (whole-model GLM, $\chi^2 = 8.356$, df = 2, $p = 0.015$), and generation 15 (whole-model GLM, $\chi^2 = 18.971$, df = 2, $p < 0.0001$). Contrasts revealed a significant

difference when medium size class females were compared to large-bodied males in generation 6 ($p = 0.003$), generation 10 ($p = 0.006$), and generation 15 ($p < 0.0001$). No EE-LHm females from medium treatments showed significant differences in sub-chamber associations in any generation (whole-model GLMs, all $p > 0.1$). In large treatments (Figures 29-32), EE-IV females showed significant differences in sub-chamber associations during generation 2 (whole-model GLM, $\chi^2 = 6.333$, df = 2, $p = 0.042$) and generation 20 (whole-model GLM, $\chi^2 = 9.227$, df = 2, $p = 0.009$). Contrasts revealed a significant difference when large size class females were compared to medium and small-bodied males pooled together in generation 2 ($p = 0.021$), while no significant difference was found in generation 20 ($p = 0.254$). No EE-LHm females from large treatments showed significant differences in sub-chamber associations at any generation (whole-model GLMs, all $p > 0.1$).

3.4 Discussion

The maintenance of variance in potentially costly female mating preferences has been a subject of intense research in the last two decades, with three main mechanisms of maintenance having been proposed. First, that preferences are maintained by direct selection due to direct benefits which increase female survival or fecundity. Second, that preferences are maintained by indirect selection due to genetic benefits that increase offspring fitness. Third, that preferences are maintained as a consequence of natural selection acting on various female sensory modalities unrelated to mate choice (e.g. ability to evade predators or acquire resources) (Jennions and Petrie, 1997). Thus, the main goals of this study were to investigate if females possessed additive genetic

variation in mate preference for body size and, consequently, if enough variation was present to allow for artificial selection to occur.

Several of my experimentally evolved lines appear to have responded to artificial selection on female mating preference for male body size. Analysis of eye phenotype ratios using binomial exact tests showed a measure of consistency among 4 treatments that produced a significant result; both EE-IV and EE-LHm small treatments (i.e. selection against small male body size) had significantly fewer bwD flies present in subsequent generations than would be expected to occur by chance over the duration of the study. These results agreed with my initial hypothesis, as I surmised that artificial selection against a given male body size (which I applied through the use of bwD males of the appropriate body size class) would result in simultaneous selection against the bwD phenotype over time. Only a single treatment, IV-M1, showed a significant difference in the direction opposite of that which I predicted (that is, significantly more brown-eyed flies were present in each generation than was expected through chance alone). That we observed the opposite of my predicted wild-type to bwD fly ratio in one of the medium body size treatments may suggest that other factors unrelated to the artificial selection can influence a population. Both variation in larval food quality and increased larval competition have been previously shown to decrease body size in *D. melanogaster* (Alpatov, 1932; Rice *et al*., 2005) and may potentially contributed to the observed data, despite my best efforts to monitor and control each of these variables. That I did not observe a significant response in IV-M2 or any of the LHm medium treatments was puzzling (Table 1), but may possibly be due to the different genetic histories of each of the founding stocks. In the other treatments where I also found no significant

differences in bwD to wild type ratios may suggest that stabilizing selection around a mean number of bwD flies has occurred, and that further effect selection against body size may be less effective as time passed.

Mean body size data compared using Dunnett's tests (using Generation 0 as the control group) revealed several differences in the direction of artificial selection between EE-IV and EE-LHm populations and treatments for each sex. In both the EE-IV and EE-LHm large body size treatments, mean body size generally decreased over time; however, the decreases occurred at different generations during the experiment for each treatment and sex. In EE-IV large treatments, male flies showed significant decreases in mean body in the initial generations of the experiment (gen 2 – gen 13), while female flies showed significant decreases throughout the duration of the experiment (see Figures 9 and 10). In EE-LHm large treatments, male flies significantly decreased in mean body size during the middle to late generations, with female flies responding similarly. That both IV and LHm males and females responded similarly in this treatment to selection against preference for large body size agrees with previous experimental evolution work done that reported artificial selection on an attractive male trait resulted in correlated changes in mating preferences (Houde, 1994; Wilkinson and Reillo, 1994). For EE-IV and EE-LHm medium body size treatments, both populations responded relatively similarly, showing significant decreases in mean body size, with a notable exception in one of IV-M1 males. Males from IV-M1 treatment were the only flies to show significant increase in mean body size, though this only occurred during two early generations (Figure 13a). IV-M2 males significantly decreased in mean body size at only generations 2, 11, and 12 (Figure 13b), while both IV-M1 and IV-M2 females both showed

significant decreases centralized around generations 12-18 (Figure 14a and 14b, respectively). EE-LHm medium treatment females showed significant decreases in mean body size in almost every generation except in generations 2-8 (Figure 16a and 16b), while EE-LHm medium treatment males showed significant decreases in mean body size in a vast majority of generations (Figure 15a and 15b). These results were surprising, as I expected mean body size to increase in both sexes in response to artificial selection against medium male body size. Larger bodied *D. melanogaster* females typically demonstrate increased fecundity (Lefranc and Bundgaard, 2000), higher rates of mating (Friberg and Arnqvist, 2003), in addition to typically preferring to mate with larger bodied males (Pitnick and Garcia-Gonzalez, 2002) when compared to smaller bodied females. Similarly, larger bodied males tend to win more male-male competition events in the laboratory (Dow and von Schilcher, 1975), are more active in seeking courtship opportunities, and can move faster than smaller males, allowing them to more easily track females during courtship (Partridge *et al.*, 1987b). Thus, I surmised that larger males should be sexually selected for when compared to males of other body size phenotypes. That I did not observe this may be due to several factors. Males outnumbered females by ~20% in all treatments, since I used additional bwD males to act as the source of selection pressure (see "3.2 Materials and Methods" for details). By biasing the operation sex ratio toward males, larger males likely would have had greater opportunity to successfully mate with high fitness females (i.e. larger bodied females) than smaller males did. However, female *D. melanogaster* have been observed to suffer direct negative fitness costs from mating with large males, showing reduced lifespan and an increased aging rate (Friberg and Arnqvist, 2003). Persistent male courtship has also been

demonstrated to directly harm females (Linder and Rice, 2005; Stewart *et al.*, 2005), in addition to incurring further negative fitness consequences on females from toxic compounds present in seminal fluid (Lung *et al.* 2002). Given that harm to females has been observed to positively correlate with male body size in *D. melanogaster* (Partridge *et al.*, 1987b) and that larger males are able to transfer more sperm to females than smaller males (Lupold *et al.*, 2010), it is conceivable that larger males transfer proportionately greater amounts of toxic compounds to females in addition to sperm and other seminal fluid components. The above mechanisms that directly affect female fitness may have all contributed to the artificial selection decreasing mean body size in EE-IV and EE-LHm medium treatments. Indeed, Pitnick (1991) found that females mated to small males had greater fitness than those mated to large males (measured as the number of adult progeny produced prior to the time where re-mating might have occurred).

To my surprise, in both EE-IV and EE-LHm small body size treatments mean body size significantly decreased in most generations, with two exceptions within each population. In EE-IV treatments, IV-S1 males showed significant decreases in body size at only generations 10-13, while IV-S2 males showed significant decreases in body size at generations 2, 6, 7, 12, & 14. EE-IV treatments for females, however, each had two generations where mean body size increased (generations 2 & 3 for IV-S1, generations 2, 3 & 5 for IV-S2), with several middle generations showing significant decreases in mean body size. In EE-LHm small treatments, mean body size significantly increased in LHm-S1 males (Figure 19a), with no significant decreases in mean body size at any point. LHm-S2 males also significantly increased in mean body size in a few early generations (generation 2, 5, & 7), though significant decreases in mean body size were also observed

in several later generations. Both LHm-S1 and LHm-S2 showed significant decreases in mean body size for females, with the exception of several early generations (Figure 20a and 20b, respectively). I expected that mean body size would increase in each treatment for both male and female flies for reasons similar to those for the medium artificial selection treatments. In this particular case, bwD male flies used were collected from the lowest portion of the body size distribution (see "3.2.2 Experimental Populations – Origin and Initial Generations" for details), ensuring that small-bodied males were consistently being used in order to exert the appropriate selection pressure. That I did not observe an increase in body size in this treatment was particularly puzzling. bwD male flies used in the small treatment were, at minimum, 41um smaller in thoracic diameter than bwD males from the medium treatment and could conceivably vary by as much as 84um. As using thoracic diameter as a proxy for body size in *D. melanogaster* has been used successfully in several studies (Long *et al.*, 2009; Long *et al.*, 2010; Turner *et al.*, 2011), I deem it unlikely that "contamination" of lower sieves with flies of larger body size occurred. In LHm males and IV females, that I observed increases in mean body size earlier generations may imply that, for a time, stereotypical "bigger is better" dynamics allowed for larger body size benefits to outweigh the apparent costs. However, as more generations passed, it is conceivable that (for reasons discussed above) selection gradually shifted back to the generation 0 mean body size in these treatments. Binomial test results for mean body size changes across each treatment further corroborate my finding that the decreasing frequency of brown-eyed flies in later generations may be due to the direct selection on female preference for male body size. I also examined whether there were equal variances in body size by testing whether there was a significant linear

increase in variance within each treatment. I only found significant increases in body size variance in IV-M1 and LHm-M2 female flies (Table 4). This was surprising, as the directional selection did not result in changes in body size variance within the majority of treatments, despite significant body size differences within all treatments in later generations when compared to generation 0. This may suggest that there was not as much genetic variation as I initially expected, or that the relationship between the artificial selection and the phenotypic response may be more complex than anticipated. If body size has a large environmental component and a small genetic component, variation in body size would therefore be less likely to occur if there were very little environmental variation.

Finally, using data collected in the behavioural assays I found significant differences between male sub-chamber associations within each population, though not for each treatment. This was encouraging and somewhat surprising, given that one of the largest studies conducted using experimental evolution on guppies (*P. reticulata*) failed to produce a significant response, even though they similarly used artificial selection on an attractive male trait and female preference for that trait (Hall *et al*., 2004). Curiously, each body size treatment always had one full population (i.e. EE-IV or EE-LHm) where females did not, in any generation, show significant changes in their behaviour. Furthermore, even in some cases where I did find a significant effect for behaviour, there were generations where the pre-planned contrasts failed to detect a significant behavioural change in the direction I predicted. While the EE-LHm small body size treatments did show significantly different measures of female sub-chamber association in generations 2 and 15, none of the pre-planned contrasts were significant. I expected

that if female preference for small-bodied males was being selected against, this would manifest in a detectable increase in sub-chamber association with both "medium" and "large"-bodied males. The reason for this is unknown; despite the fitness costs females incur by mating with small-bodied males (Lupold *et al*., 2010), they still appeared to prefer associating with them. Thus, it is conceivable that the imposed costs were less than the actual costs of associating with large-bodied males. In contrast to the small body size treatment, the medium body size treatment showed no significant responses in female behaviour in the EE-LHm population, but showed significant responses in the EE-IV population in three generations (6, 10, and 15). Additionally, each of the contrasts performed on these generations were significant, with females associating significantly more with sub-chambers that contained large-bodied males. In the context of female sub-chamber association, I expected this result due to the vast amount of literature reporting that, in both nature and the laboratory, mating males are larger on average than non-copulatory males (Partridge *et al*., 1987b; Markow, 1988; Pitnick, 1991; Andersson, 1994). However, in the context of the earlier mean body size results (where mean body size decreased in medium body size treatments), the large-bodied males used in the behavioural assays were sampled from wild-type stocks, and thus may not had the same genetic constraints limiting their traits that indirectly allow a female to sample potential mates (e.g. cuticular hydrocarbon profile, wing song amplitude/modulation, etc). Lastly, the large body size treatment only showed significant behavioural responses in the EE-IV population for generations 2 and 20, with only generation 2 reporting significant contrast results. That a response was observed in this generation coincides with findings reported in a *P. reticulata* artificial selection experiment (Houde, 1994), whereby significant

differences on a male colour pattern on female mating preference was observed within 3 generations. However, Houde (1994) also found that preferences diverged away from the initial direction of the artificial selection pressure after the third generation, potentially due to high population density reducing the ability of females to exercise mate preference. Given that my study used and maintained 700 adult virgin male and female flies per treatment (plus an additional 75 bwD male flies), it is possible that I observed such little effect of the artificial selection for similar reasons that Houde (1994) did.

Variation in mating preferences can potentially be generated by many different processes. The amount of heritable variation, mating competition, sampling tactics used, as well as environmental constraints can all influence how preferences are expressed within a population. However, few empirical studies have directly examined if variation in preferences has a genetic component, and thus empirical evidence is scarce. My assays provide some evidence that *D. melanogaster* females possess significant variation in mate preferences for male body size. That artificial selection was able to be successfully used to directly select on a female preference, in several independent treatments, suggests that both mate preference and body size represent significant sources of variation which may be subject to sexual selection. However, that many of the treatments did not respond to selection, and that both of the founding wild-type populations varied widely in their responses to selection, leave many questions unanswered of how mate preference variation and its associated traits are maintained (or lost) in both natural and laboratory populations.

3.5 Figures and Tables

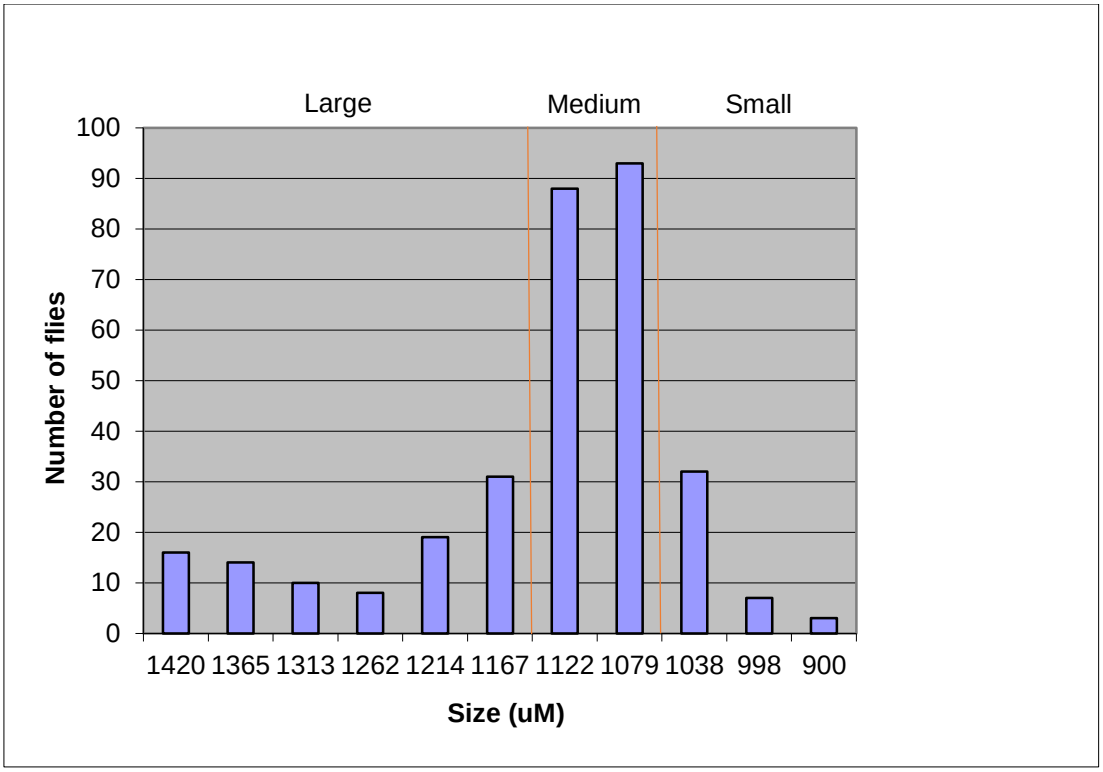

Figure 5. Histogram of thoracic diameter distribution of male IV flies compiled from 350 individuals, sorted using a sieve sorting system (see "Experimental Populations – Origin and Structure of Experiments" for details). Lines indicate separate IV-bwD size categories used in EE lines.

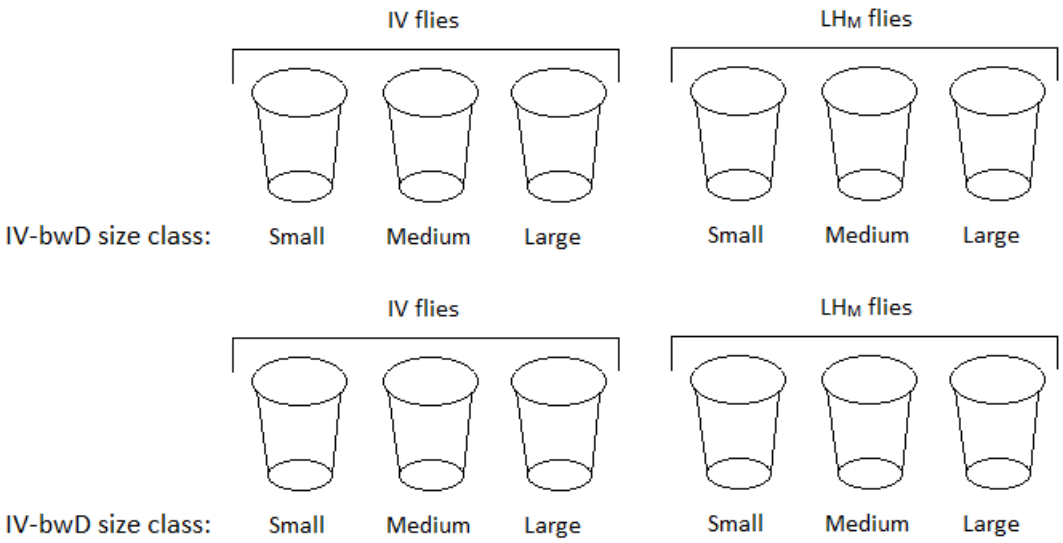

Figure 6. Pairing of IV-bwD male size classes to IV and LH_m experimental populations. Each IV-bwD size class treatment was replicated twice in all IV and LHm experimental populations.

Figure 7. A typical population cage in which EE males and females and IV-bwD males of specific size classes interact (see "3.2.2 Experimental Populations – Origin and Structure of Experiments" for details). Media can be introduced and removed via the fabric sleeve.

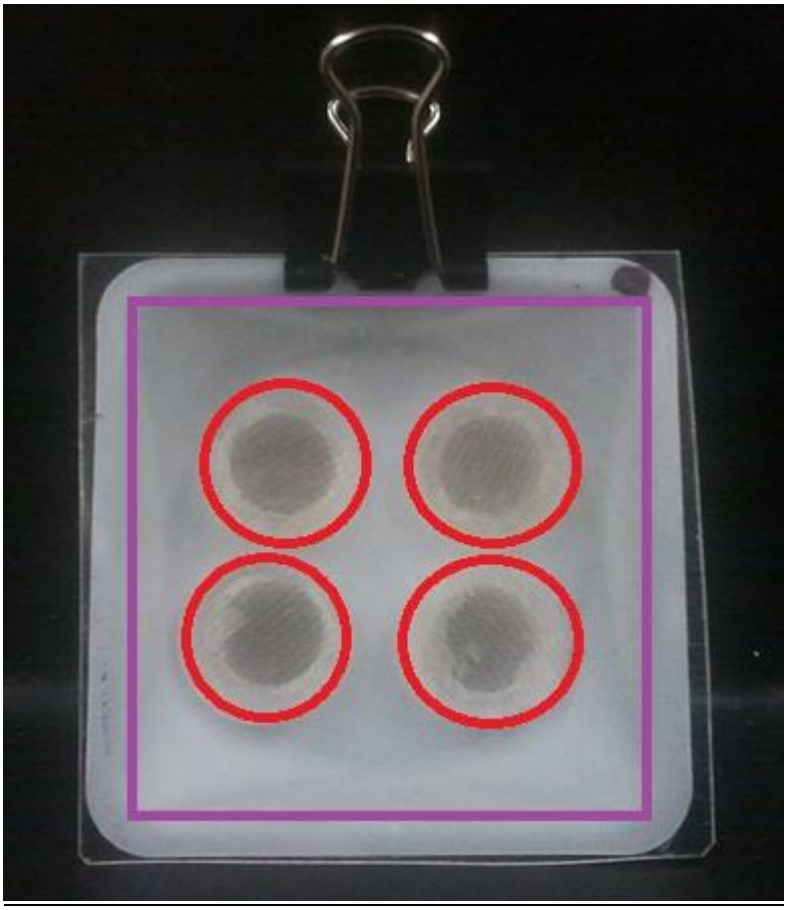

Figure 8. Female preference chamber as seen from the front, with sub-chambers clearly visible. The top right dot represents where the corner sub-chamber containing only media was located, which allowed for easy re-orientation of each chamber to account for spatial effects. Red circles indicate where a "region of interest" (ROI) was designated in VideoFly, with each ROI tallying the number of times a female was found to reside within it for the duration of the assay. The purple square indicates the overall area of motion-tracking.

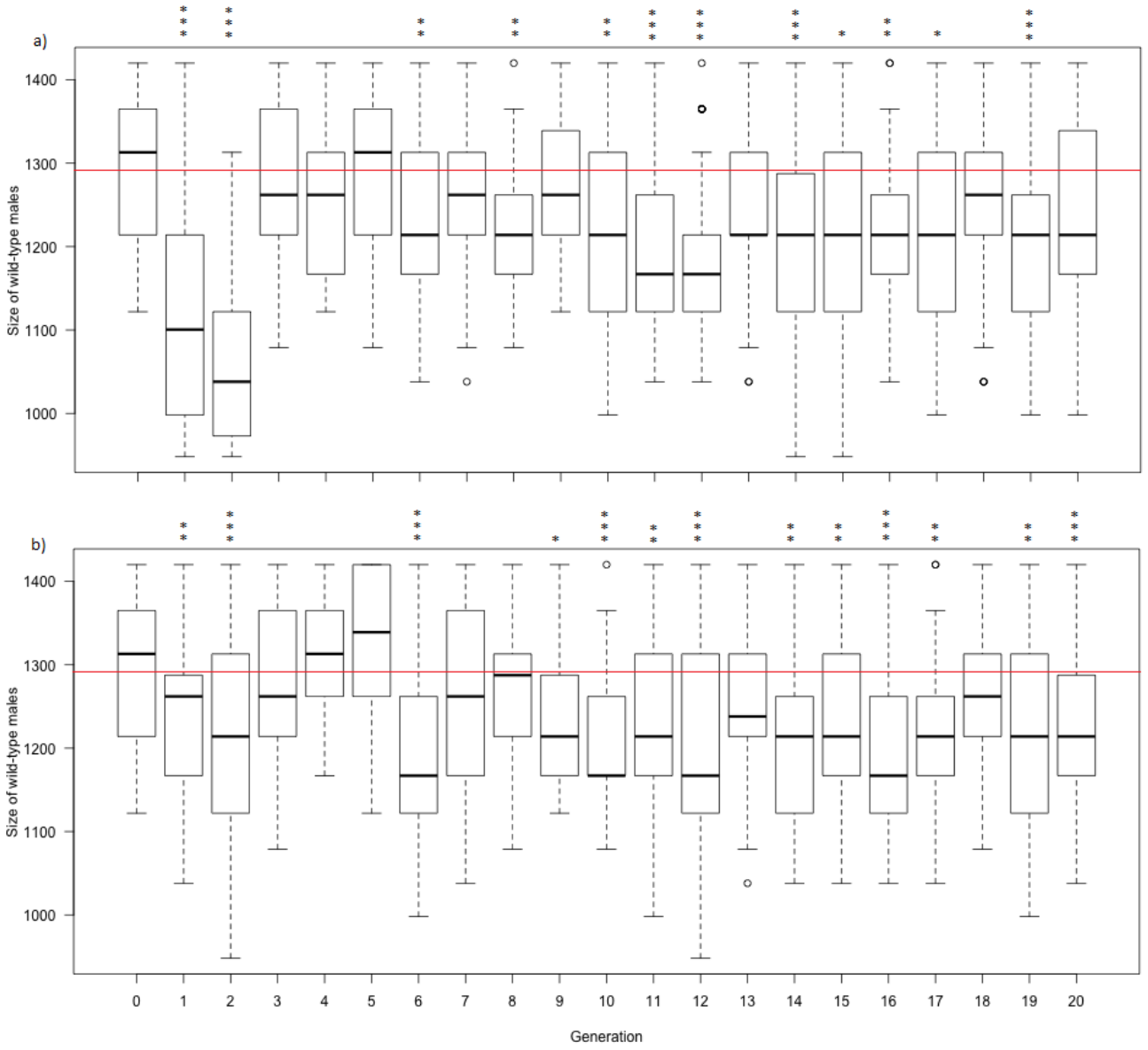

Figure 9. Boxplots of thoracic diameters (in µm) by generation for: a) IV-L1 males and b) IV-L2 males. Bold horizontal lines represent the median, with the box representing the 25th and 75th percentiles, the whiskers representing 1.5 times the interquartile range, and outliers represented by open circles. Asterisks indicate generations where the mean differed significantly from the generation 0 mean (red line) using Dunnett's tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

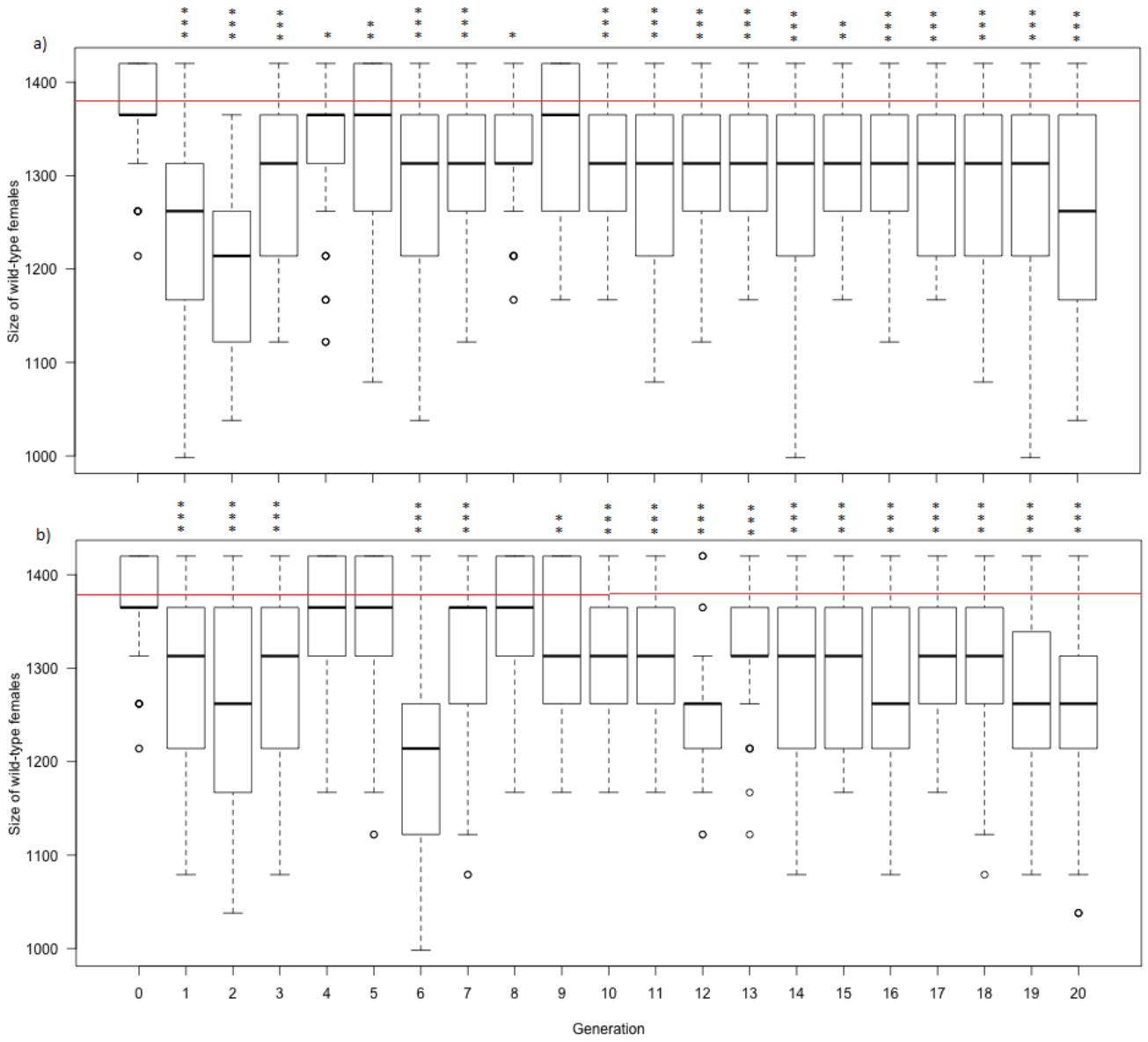

Figure 10. Boxplots of thoracic diameters (in µm) by generation for: a) IV-L1 females and b) IV-L2 females. Bold horizontal lines represent the median, with the box representing the 25th and 75th percentiles, the whiskers representing 1.5 times the interquartile range, and outliers represented by open circles. Asterisks indicate generations where the mean differed significantly from the generation 0 mean (red line) using Dunnett's tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

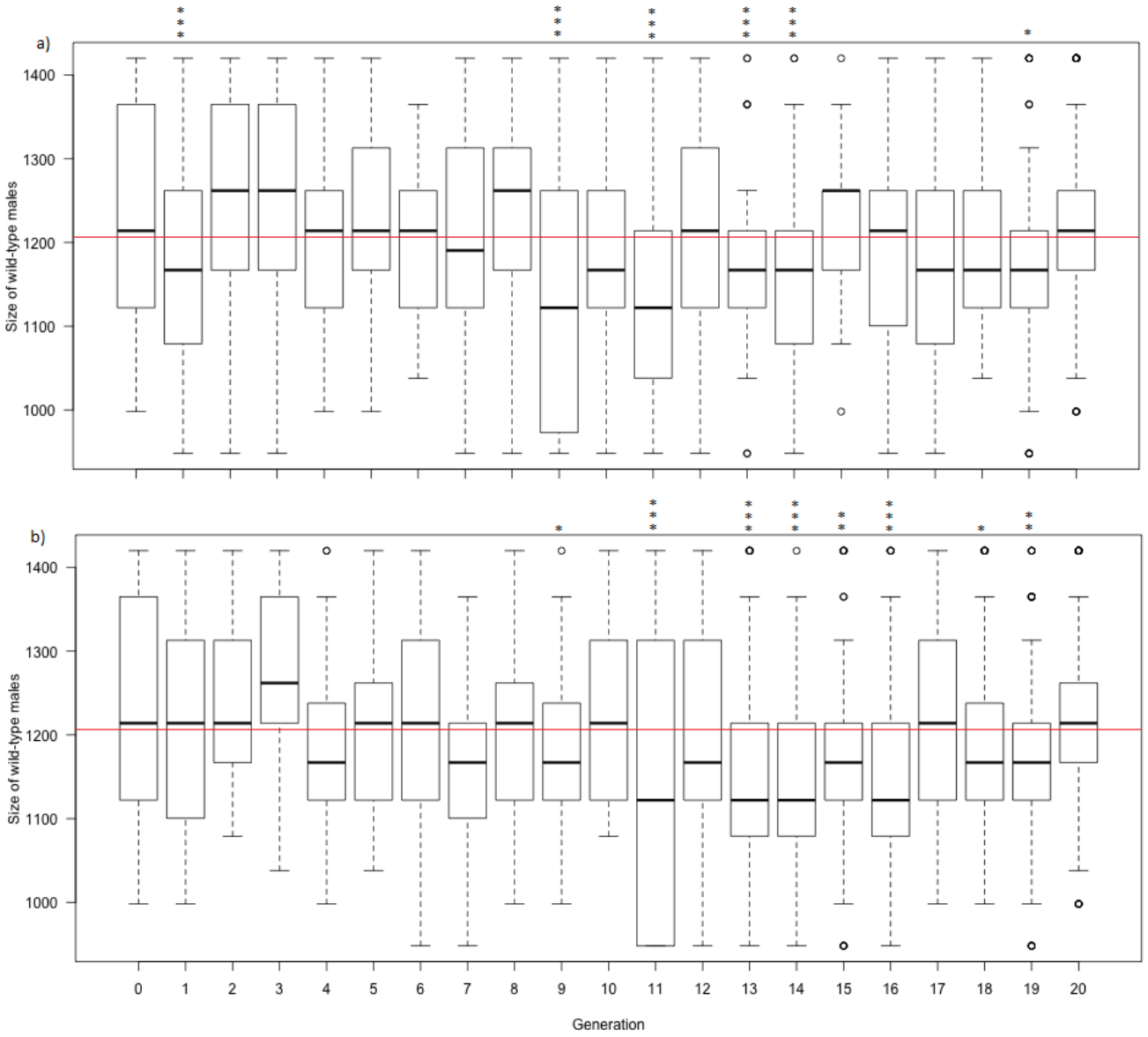

Figure 11. Boxplots of thoracic diameters (in µm) by generation for: a) LHm-L1 males and b) LHm-L2 males. Bold horizontal lines represent the median, with the box representing the 25th and 75th percentiles, the whiskers representing 1.5 times the interquartile range, and outliers represented by open circles. Asterisks indicate generations where the mean differed significantly from the generation 0 mean (red line) using Dunnett's tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

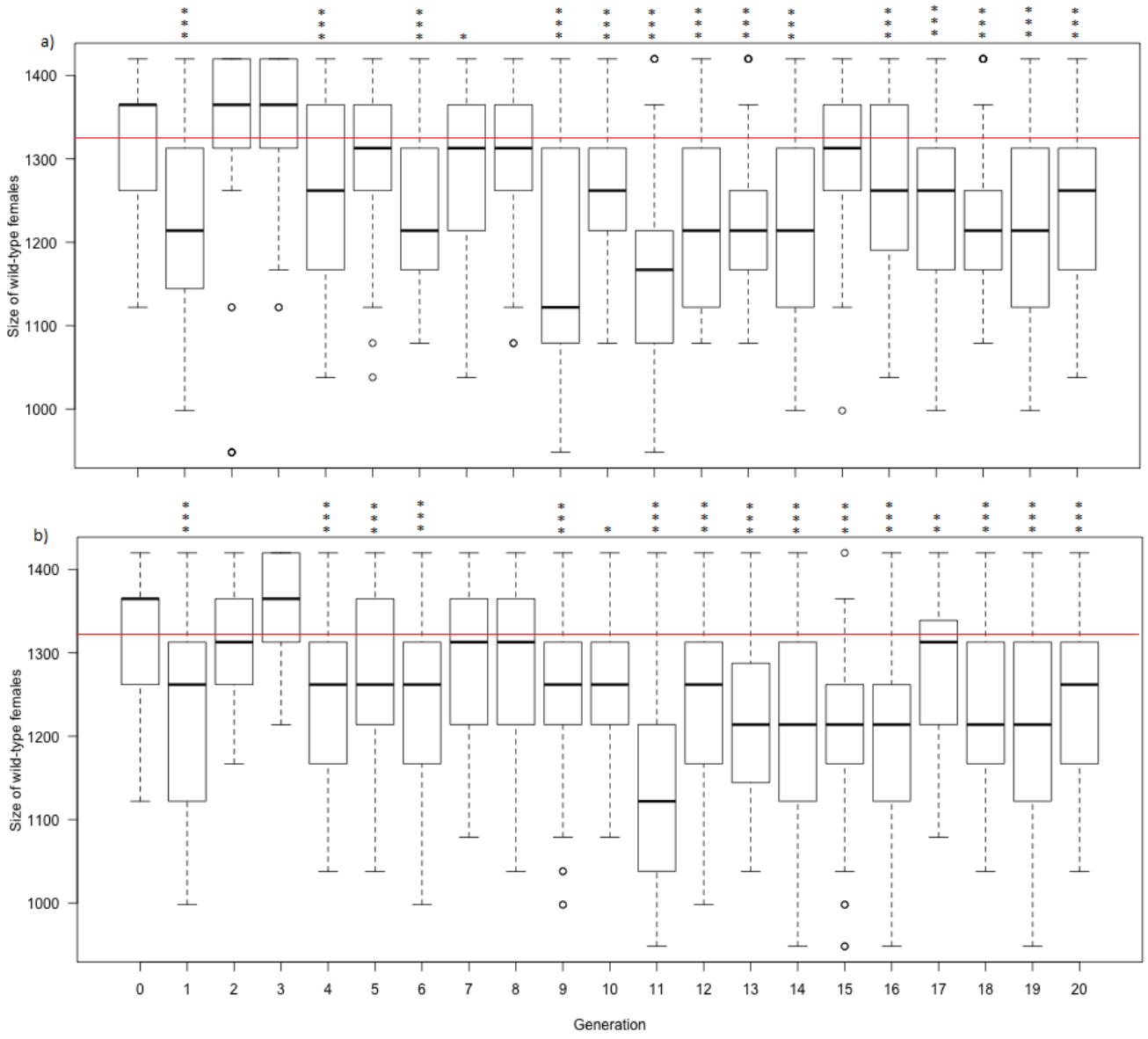

Figure 12. Boxplots of thoracic diameters (in µm) by generation for: a) LHm-L1 females and b) LHm-L2 females. Bold horizontal lines represent the median, with the box representing the 25th and 75th percentiles, the whiskers representing 1.5 times the interquartile range, and outliers represented by open circles. Asterisks indicate generations where the mean differed significantly from the generation 0 mean (red line) using Dunnett's tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

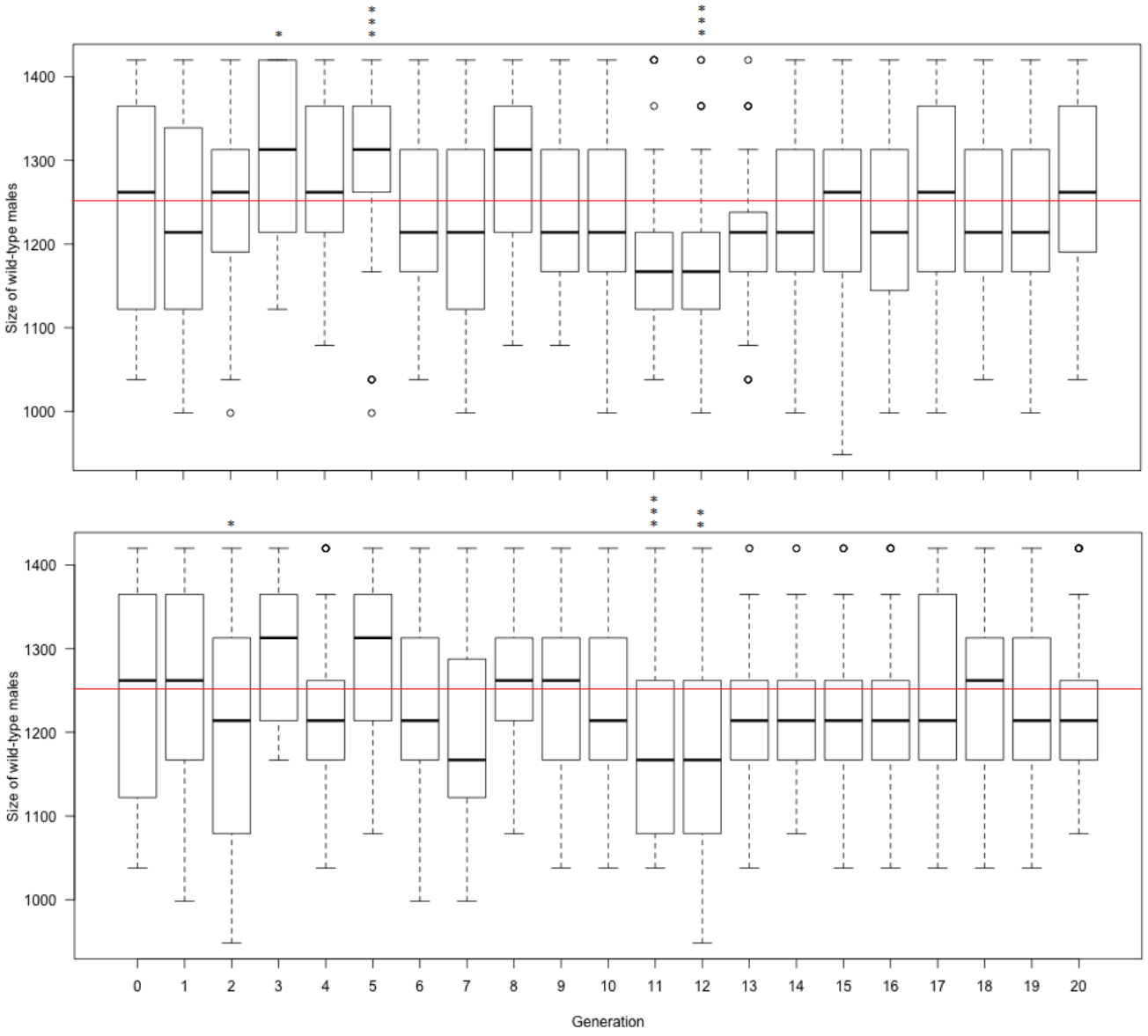

Figure 13. Boxplots of thoracic diameters (in µm) by generation for: a) IV-M1 males and b) IV-M2 males. Bold horizontal lines represent the median, with the box representing the 25th and 75th percentiles, the whiskers 1.5 times representing the interquartile range, and outliers represented by open circles. Asterisks indicate generations where the mean differed significantly from the generation 0 mean (red line) using Dunnett's tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

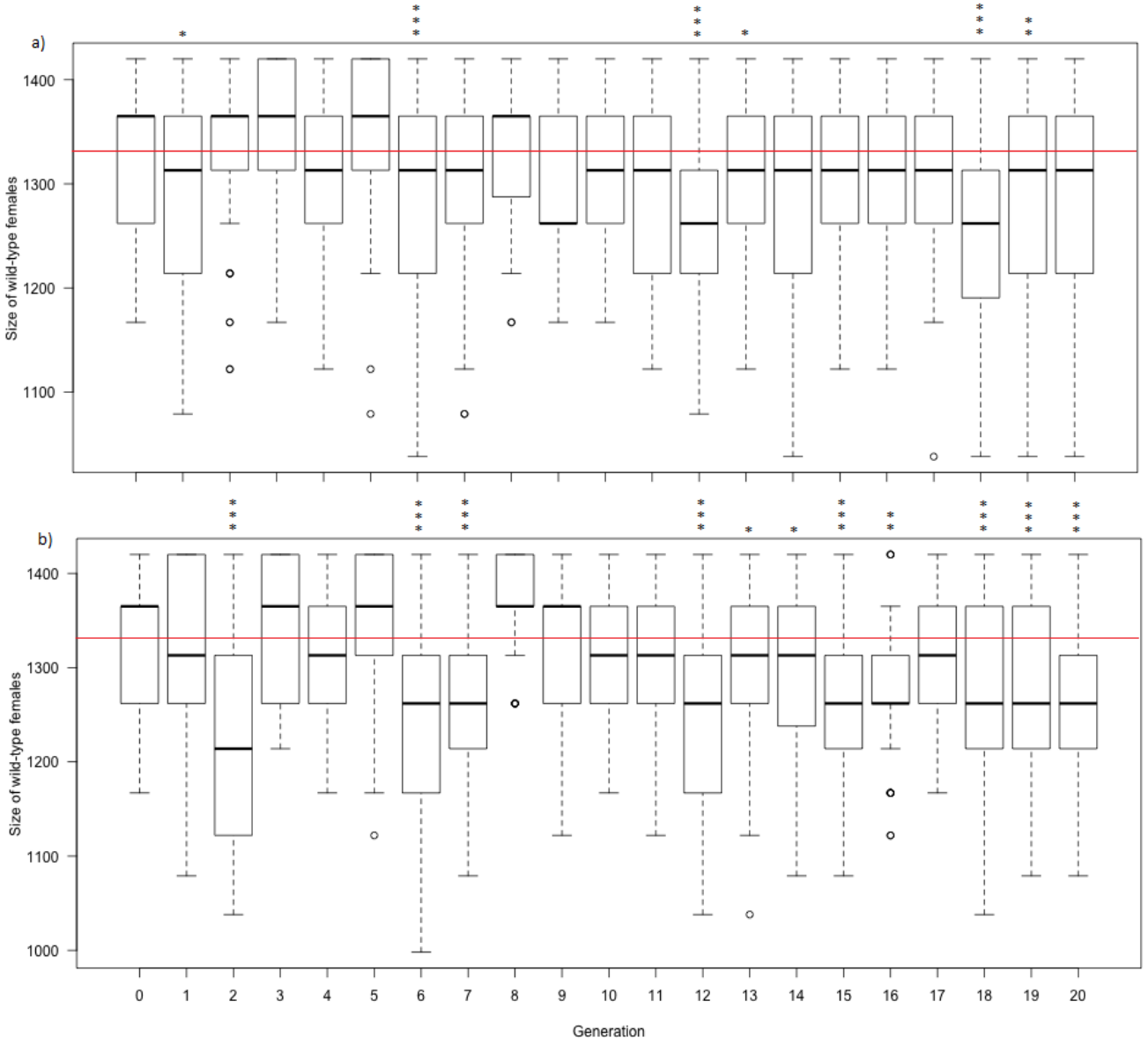

Figure 14. Boxplots of thoracic diameters (in µm) by generation for: a) IV-M1 females and b) IV-M2 females. Bold horizontal lines represent the median, with the box representing the 25th and 75th percentiles, the whiskers representing 1.5 times the interquartile range, and outliers represented by open circles. Asterisks indicate generations where the mean differed significantly from the generation 0 mean (red line) using Dunnett's tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

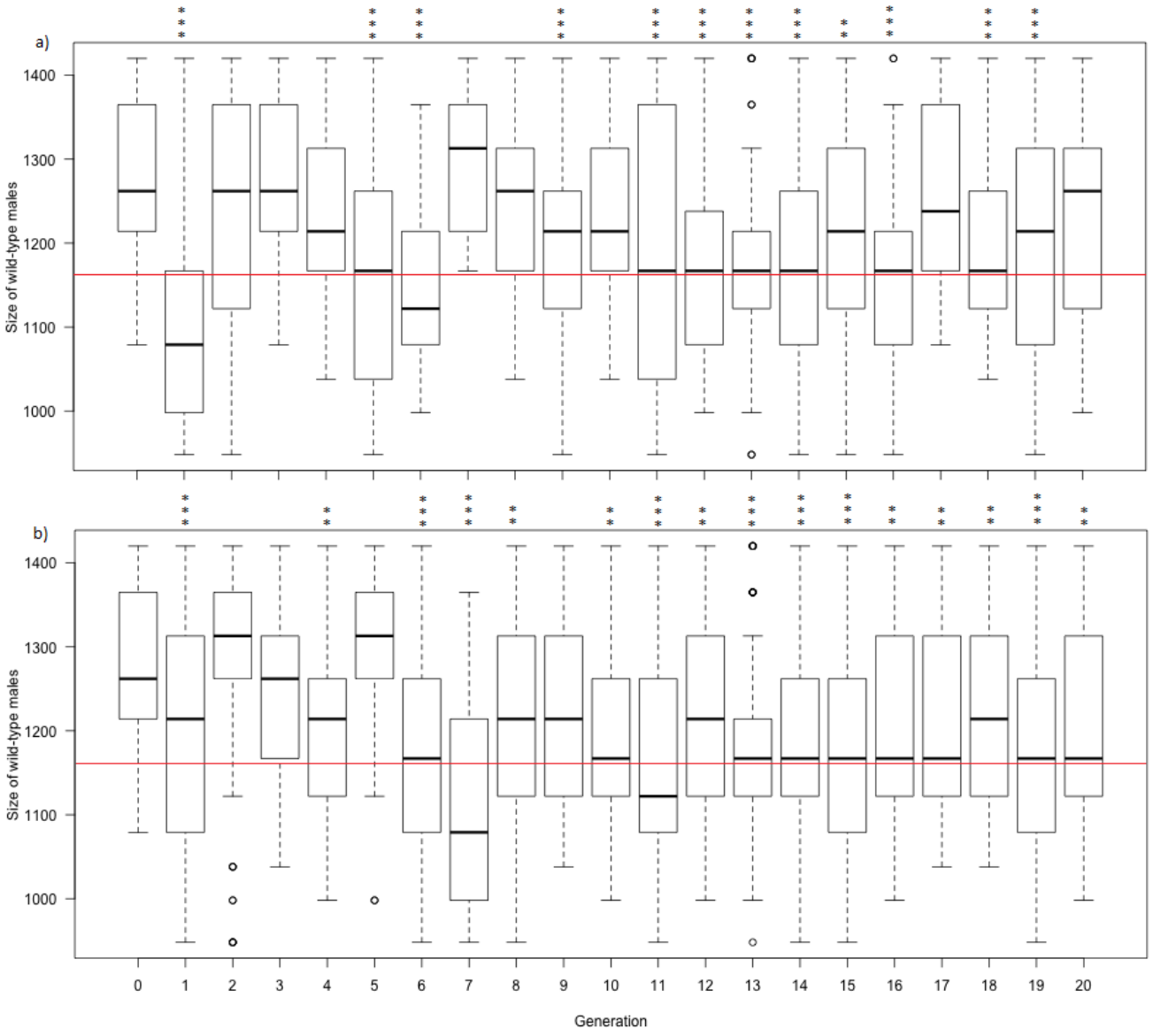

Figure 15. Boxplots of thoracic diameters (in µm) by generation for: a) LHm-M1 males and b) LHm-M2 males. Bold horizontal lines represent the median, with the box representing the 25th and 75th percentiles, the whiskers 1.5 times representing the interquartile range, and outliers represented by open circles. Asterisks indicate generations where the mean differed significantly from the generation 0 mean (red line) using Dunnett's tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

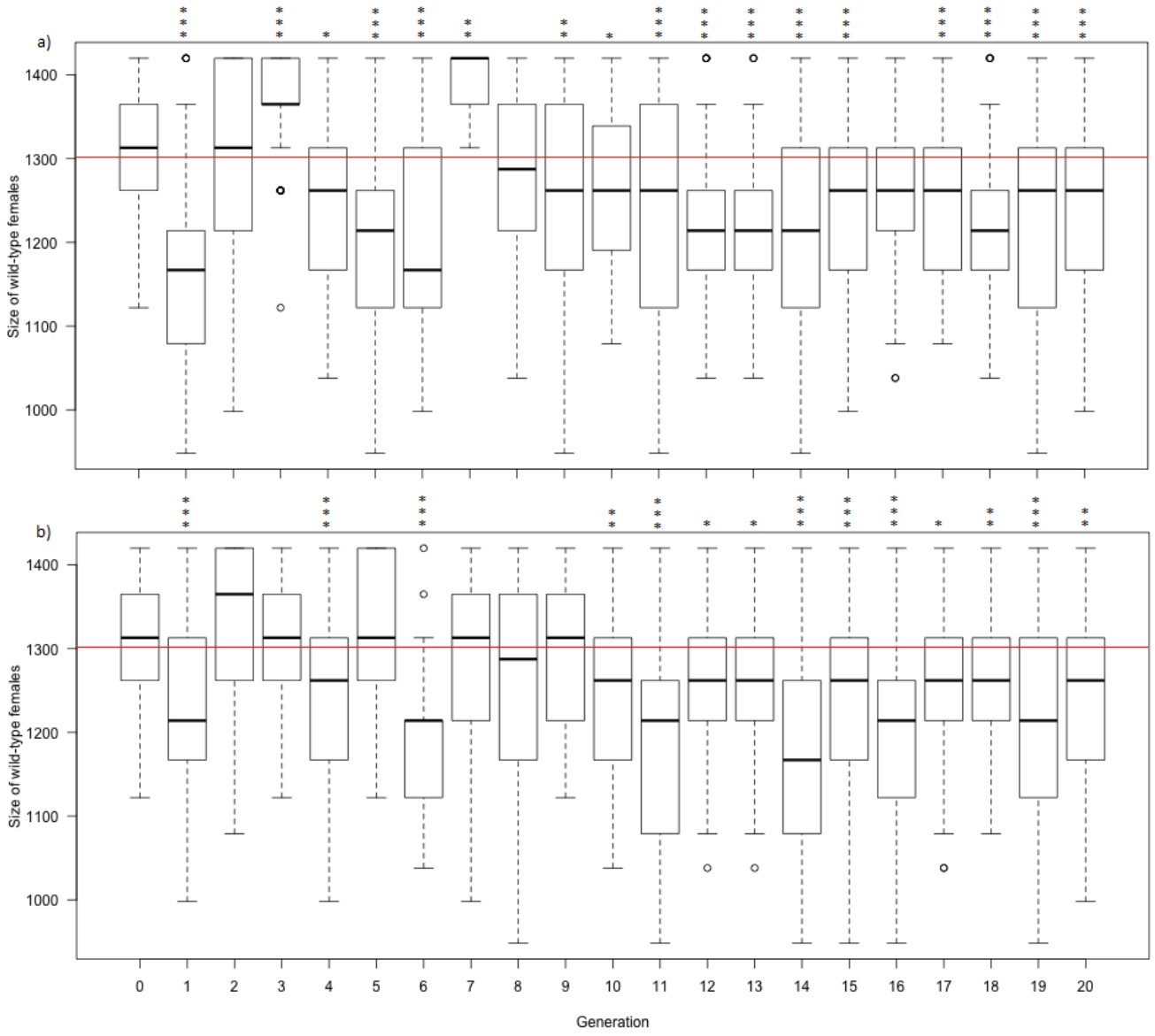

Figure 16. Boxplots of thoracic diameters (in µm) by generation for: a) LHm-M1 females and b) LHm-M2 females. Bold horizontal lines represent the median, with the box representing the 25th and 75th percentiles, the whiskers 1.5 times representing the interquartile range, and outliers represented by open circles. Asterisks indicate generations where the mean differed significantly from the generation 0 mean (red line) using Dunnett's tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

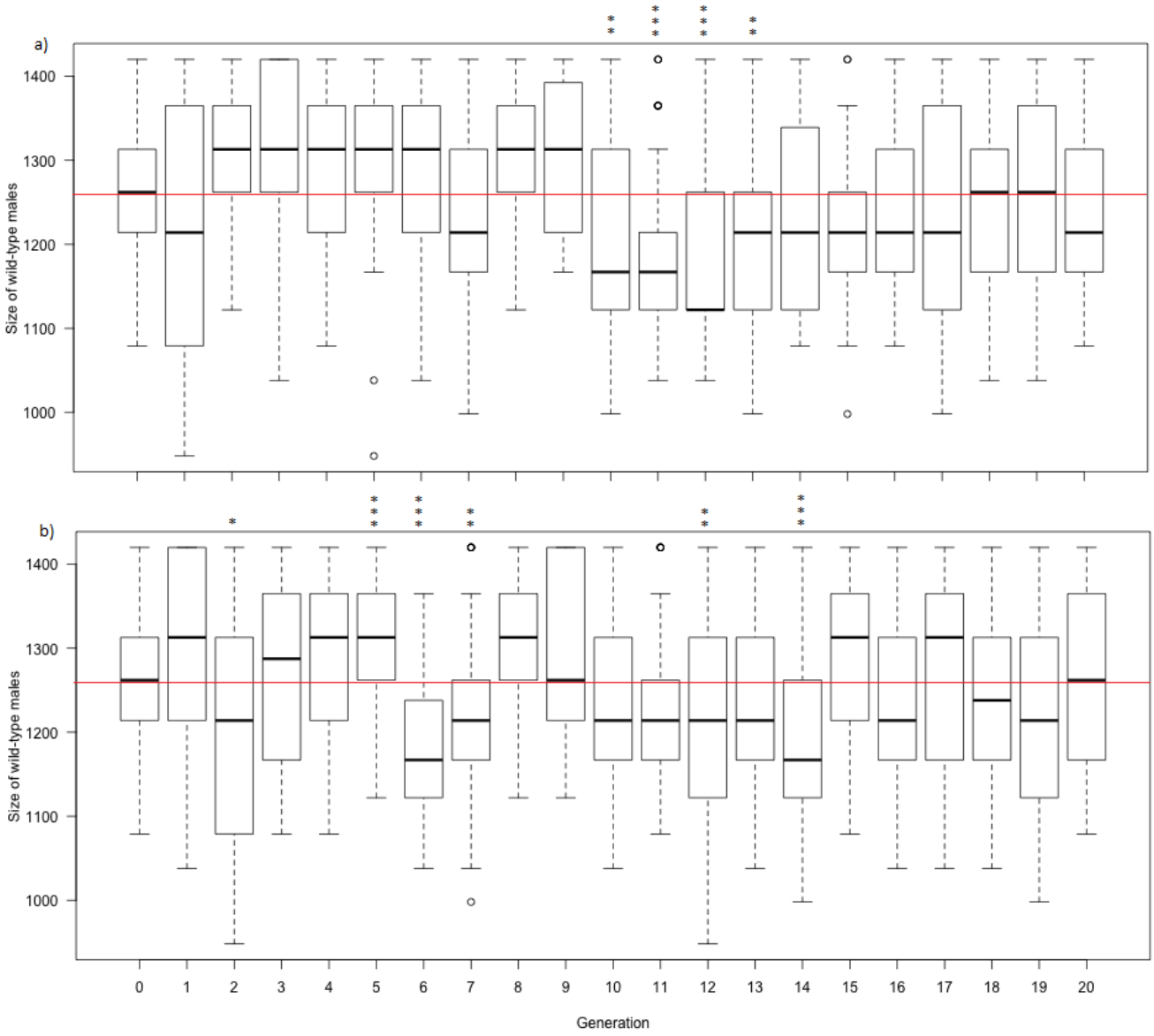

Figure 17. Boxplots of thoracic diameters (in µm) by generation for: a) IV-S1 males and b) IV-S2 males. Bold horizontal lines represent the median, with the box representing the 25th and 75th percentiles, the whiskers 1.5 times representing the interquartile range, and outliers represented by open circles. Asterisks indicate generations where the mean differed significantly from the generation 0 mean (red line) using Dunnett's tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

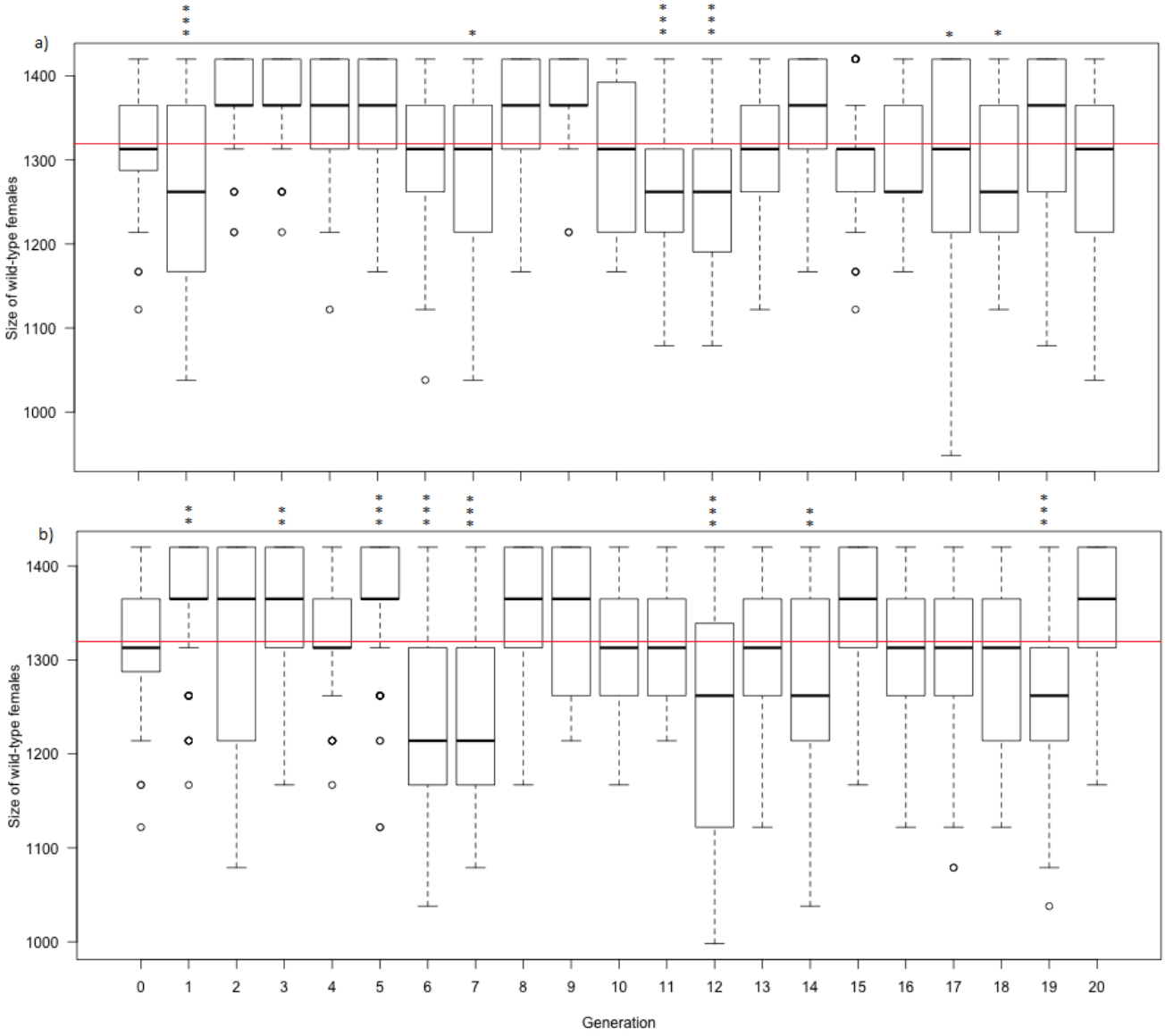

Figure 18. Boxplots of thoracic diameters (in µm) by generation for: a) IV-S1 females and b) IV-S2 females. Bold horizontal lines represent the median, with the box representing the 25th and 75th percentiles, the whiskers 1.5 times representing the interquartile range, and outliers represented by open circles. Asterisks indicate generations where the mean differed significantly from the generation 0 mean (red line) using Dunnett's tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

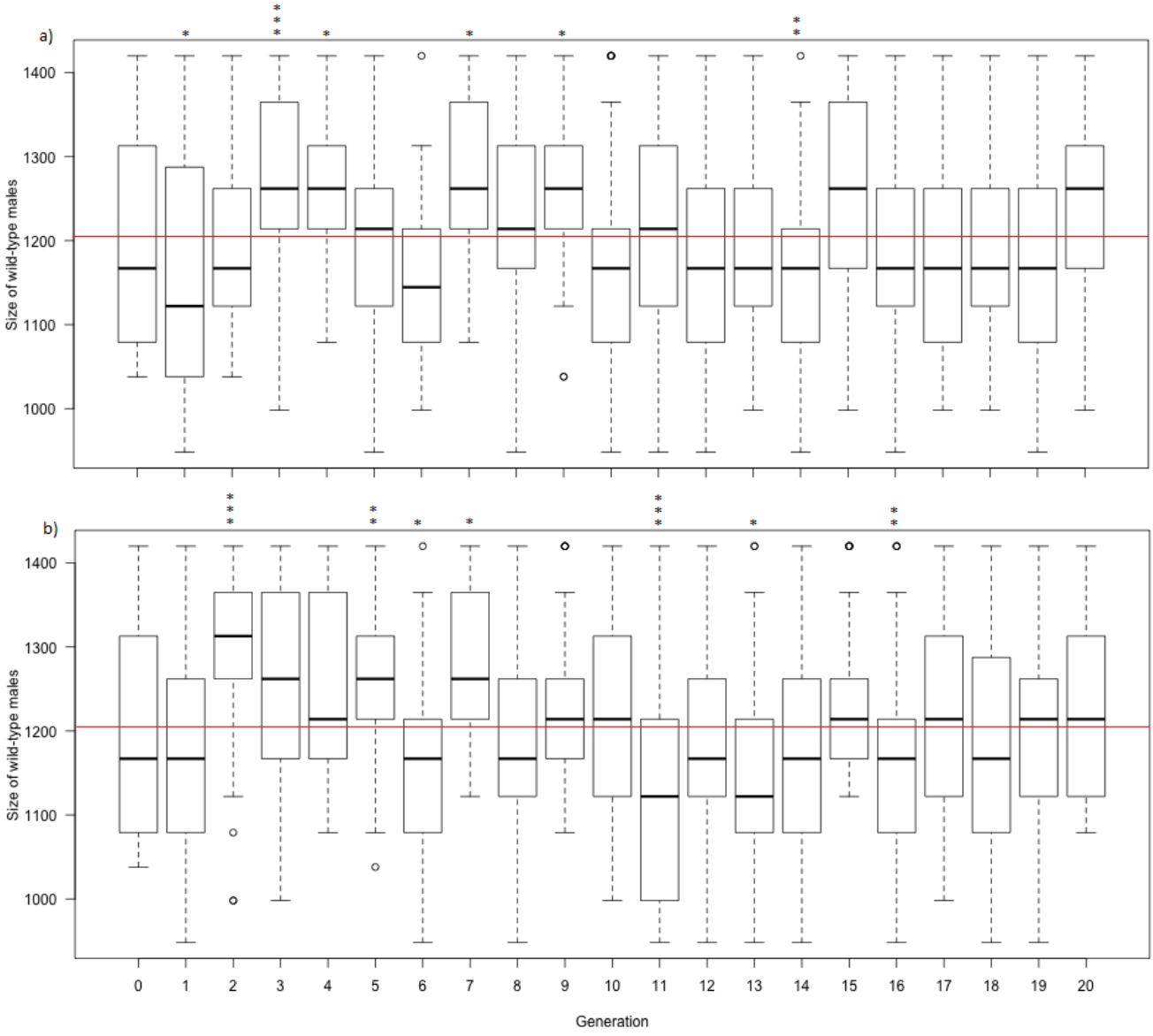

Figure 19. Boxplots of thoracic diameters (in µm) by generation for: a) LHm-S1 males and b) LHm-S2 males. Bold horizontal lines represent the median, with the box representing the 25th and 75th percentiles, the whiskers 1.5 times representing the interquartile range, and outliers represented by open circles. Asterisks indicate generations where the mean differed significantly from the generation 0 mean (red line) using Dunnett's tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

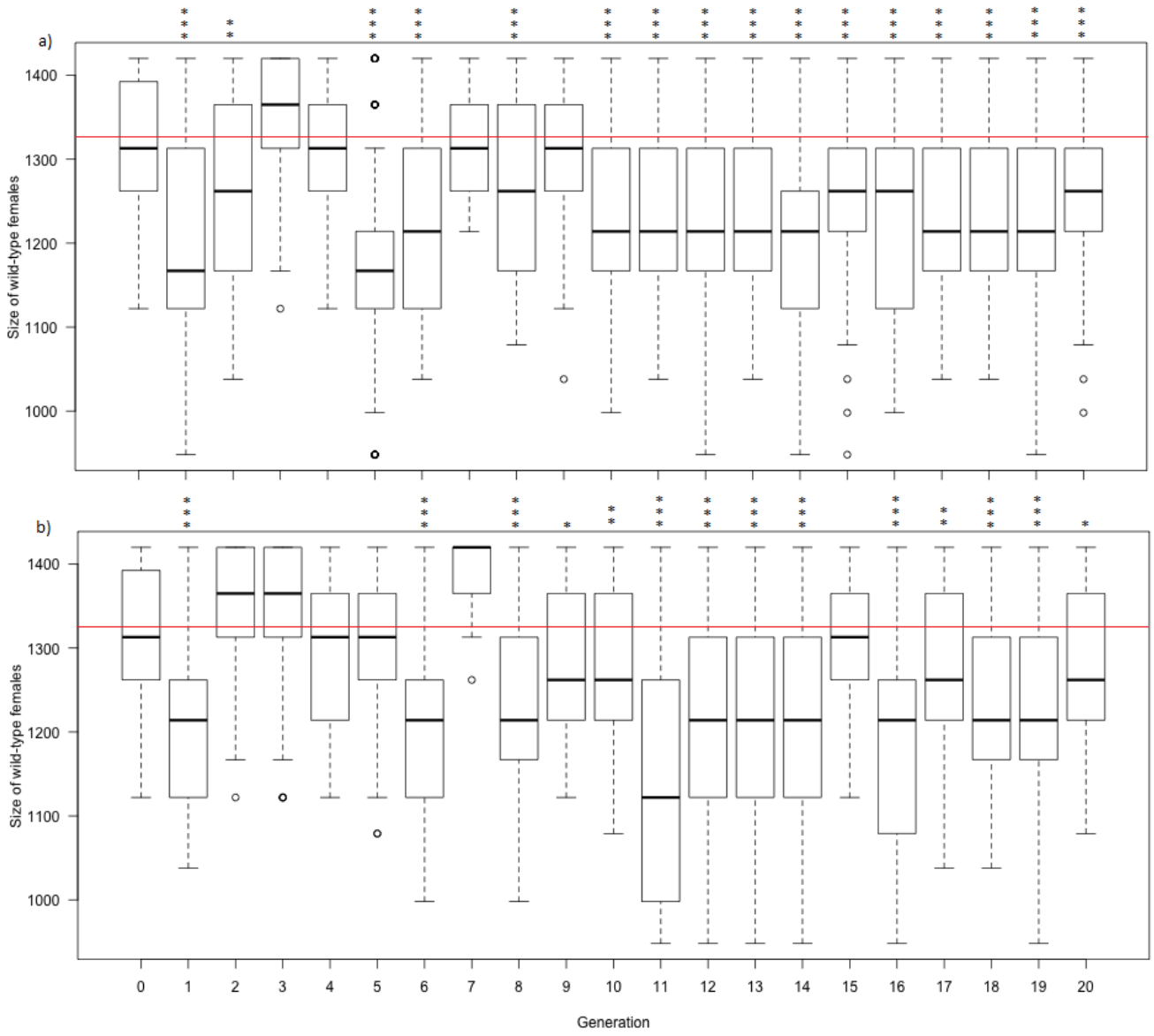

Figure 20. Boxplots of thoracic diameters (in µm) by generation for: a) LHm-S1 females and b) LHm-S2 females. Bold horizontal lines represent the median, with the box representing the 25th and 75th percentiles, the whiskers 1.5 times representing the interquartile range, and outliers represented by open circles. Asterisks indicate generations where the mean differed significantly from the generation 0 mean (red line) using Dunnett's tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

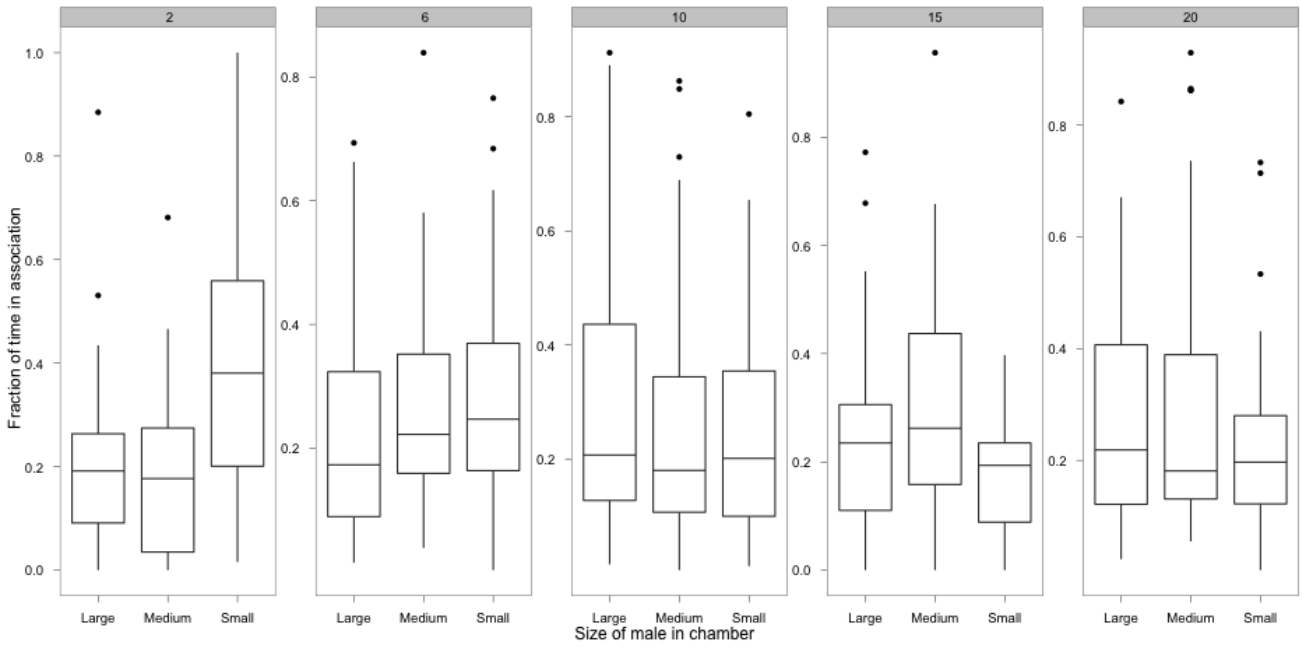

Figure 21. Boxplots of the fraction of times female flies from the LHm-S1 treatments associated with sub-chambers containing males of each body size class for each generation (indicated above each boxplot triplet). Horizontal lines represent the mean, with the box representing the 25th and 75th percentiles, the whiskers representing 1.5 times the interquartile range, and outliers represented by closed circles.

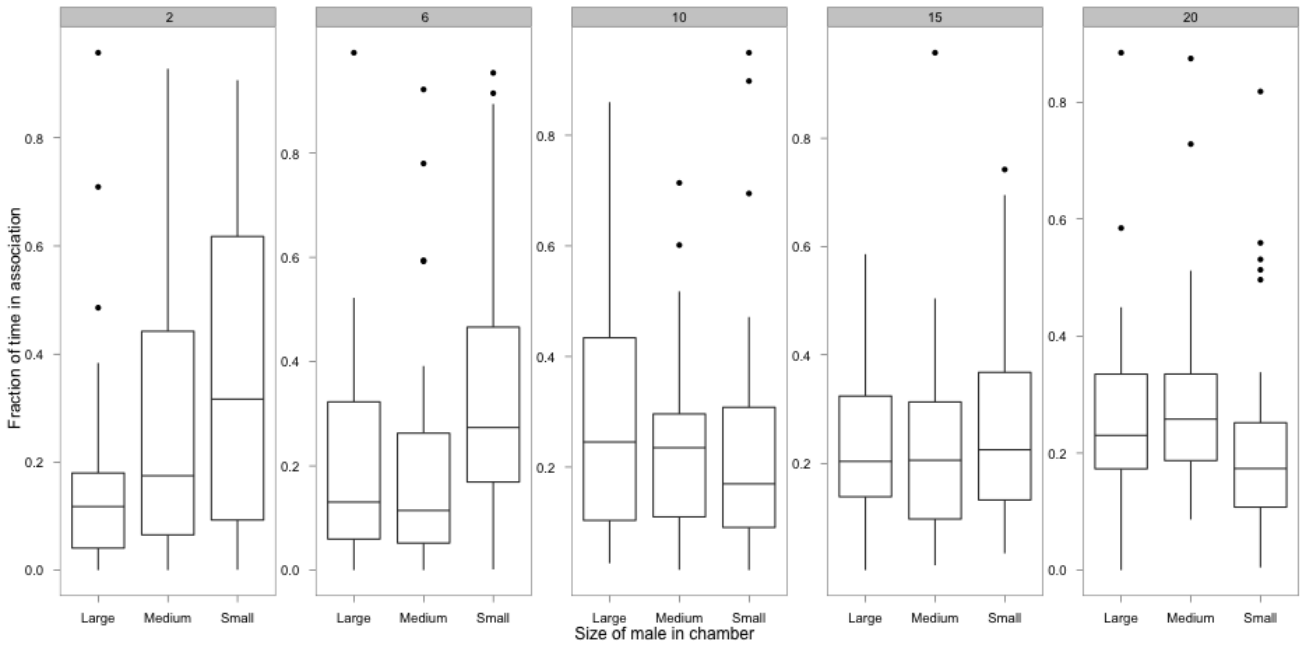

Figure 22. Boxplots of the fraction of times female flies from the LHm-S2 treatments associated with sub-chambers containing males of each body size class for each generation (indicated above each boxplot triplet). Horizontal lines represent the mean, with the box representing the 25th and 75th percentiles, the whiskers representing 1.5 times the interquartile range, and outliers represented by closed circles.

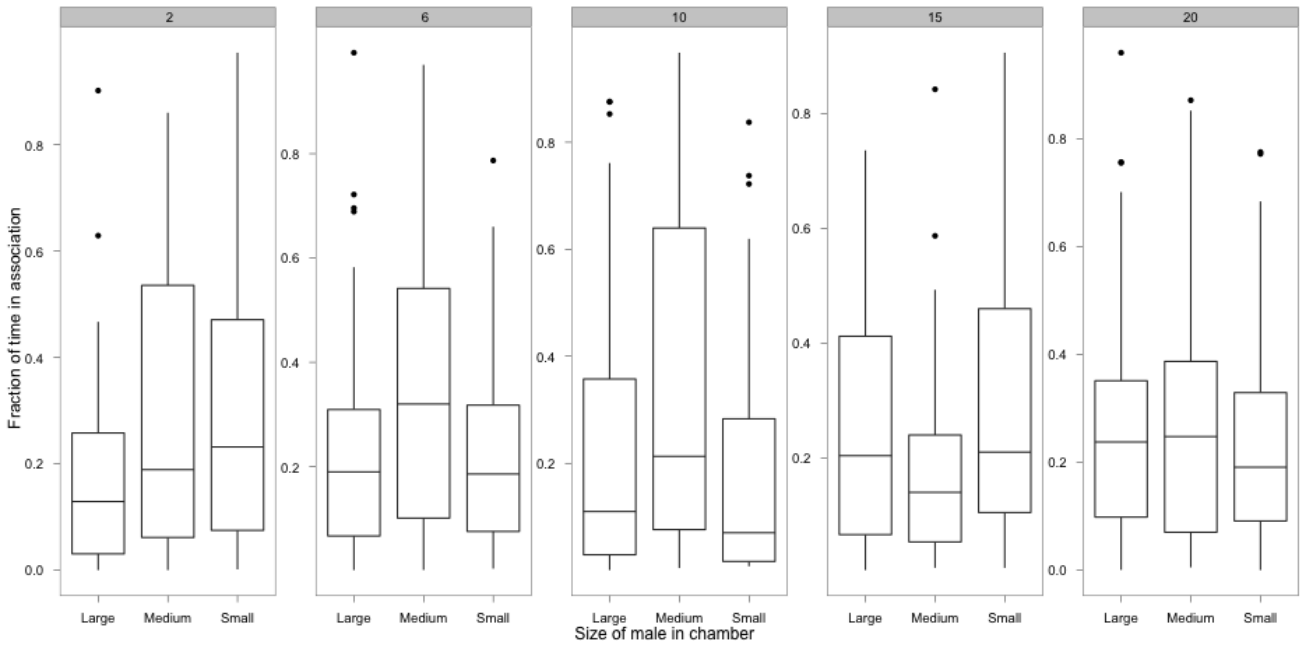

Figure 23. Boxplots of the fraction of times female flies from the IV-S1 treatments associated with sub-chambers containing males of each body size class for each generation (indicated above each boxplot triplet). Horizontal lines represent the mean, with the box representing the 25th and 75th percentiles, the whiskers representing 1.5 times the interquartile range, and outliers represented by closed circles.

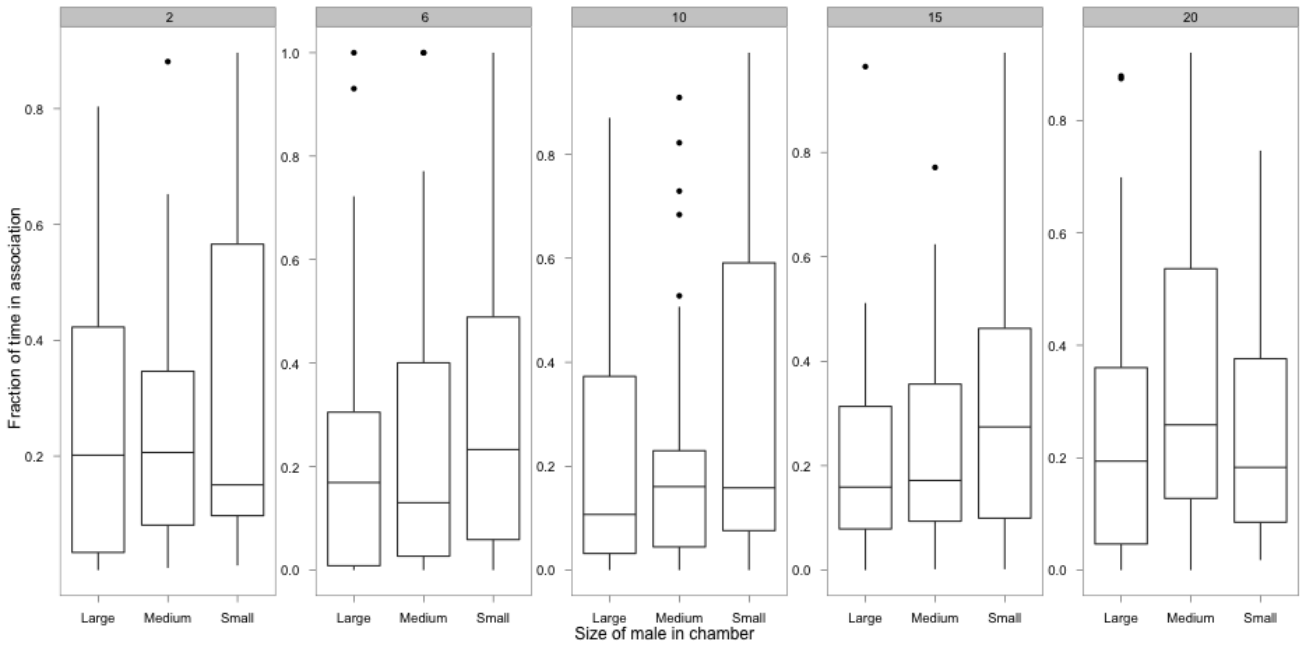

Figure 24. Boxplots of the fraction of times female flies from the IV-S2 treatments associated with sub-chambers containing males of each body size class for each generation (indicated above each boxplot triplet). Horizontal lines represent the mean, with the box representing the 25th and 75th percentiles, the whiskers representing 1.5 times the interquartile range, and outliers represented by closed circles.

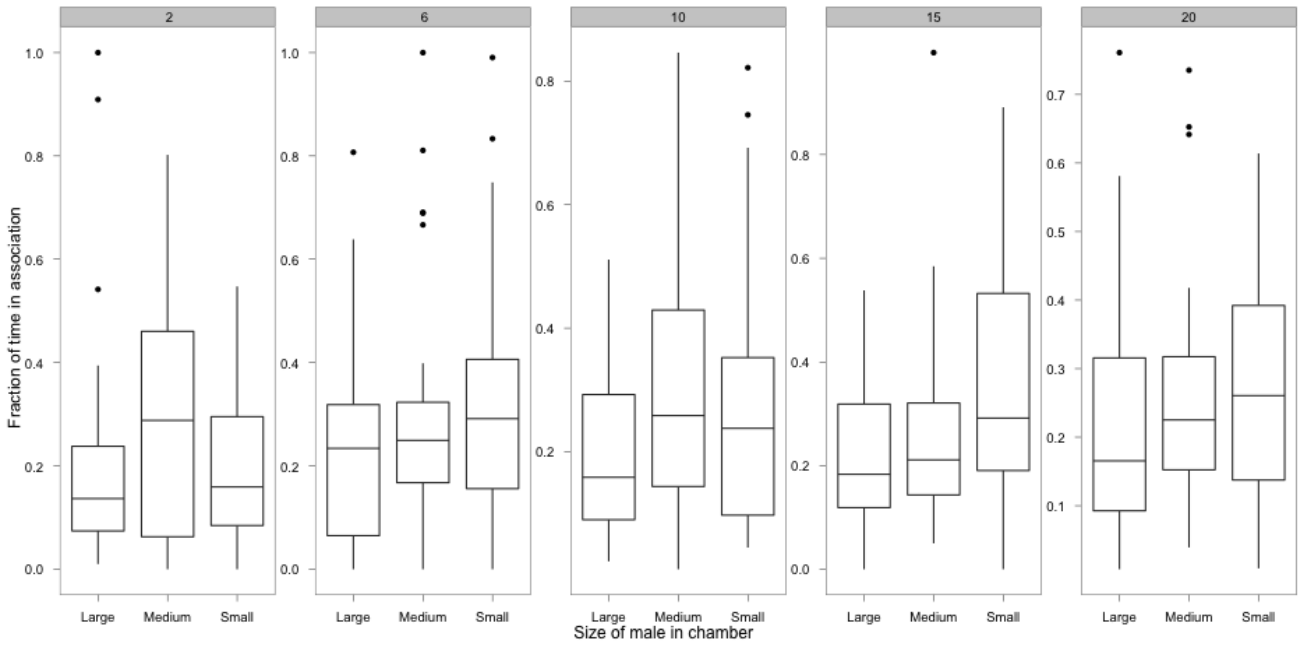

Figure 25. Boxplots of the fraction of times female flies from the LHm-M1 treatments associated with sub-chambers containing males of each body size class for each generation (indicated above each boxplot triplet). Horizontal lines represent the mean, with the box representing the 25th and 75th percentiles, the whiskers representing 1.5 times the interquartile range, and outliers represented by closed circles.

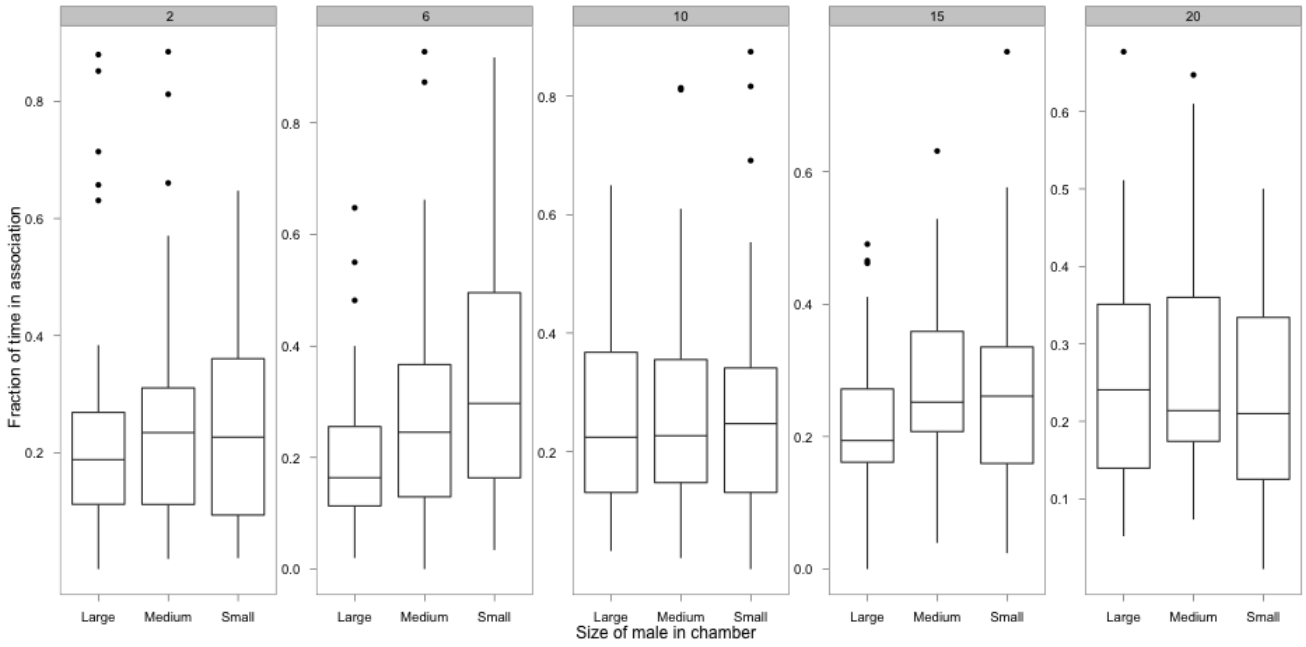

Figure 26. Boxplots of the fraction of times female flies from the LHm-M2 treatments associated with sub-chambers containing males of each body size class for each generation (indicated above each boxplot triplet). Horizontal lines represent the mean, with the box representing the 25th and 75th percentiles, the whiskers representing 1.5 times the interquartile range, and outliers represented by closed circles.

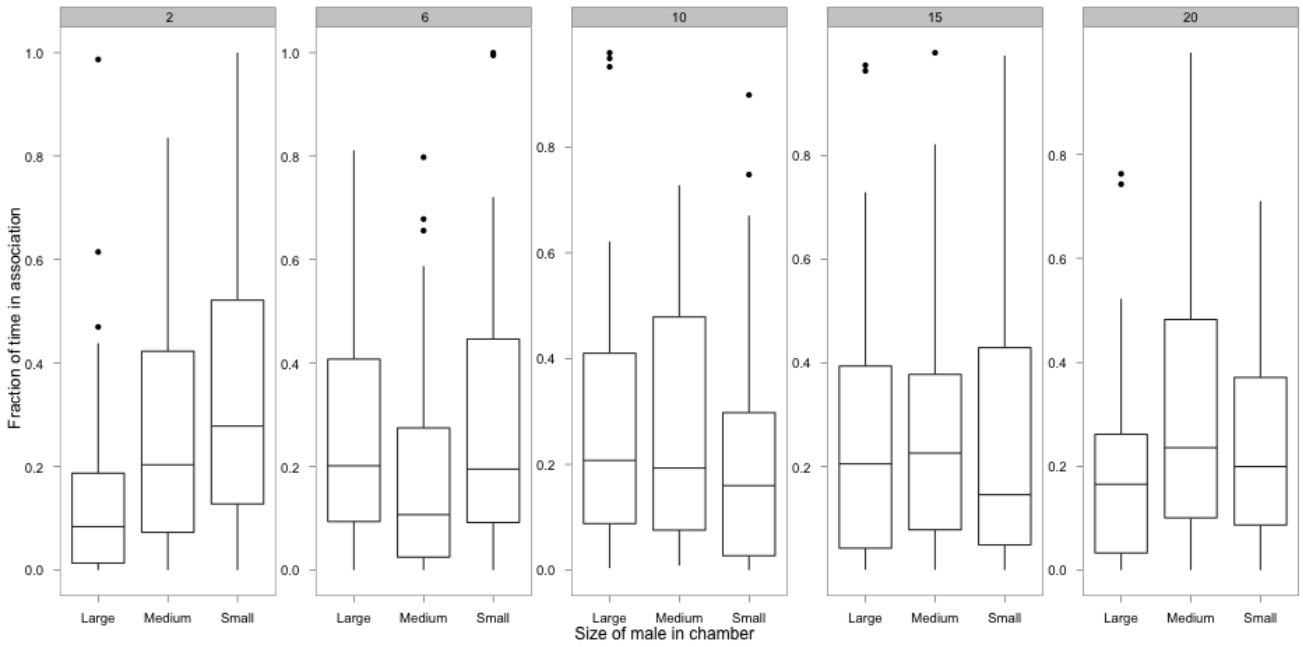

Figure 27. Boxplots of the fraction of times female flies from the IV-M1 treatments associated with sub-chambers containing males of each body size class for each generation (indicated above each boxplot triplet). Horizontal lines represent the mean, with the box representing the 25th and 75th percentiles, the whiskers representing 1.5 times the interquartile range, and outliers represented by closed circles.

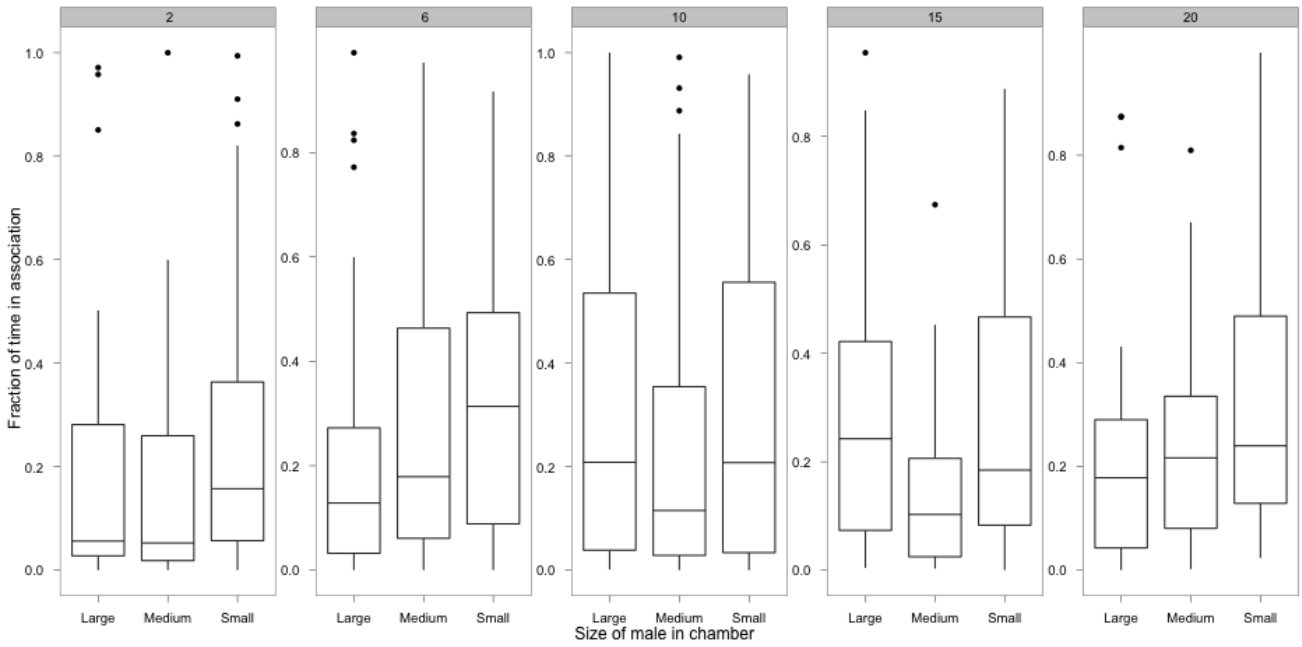

Figure 28. Boxplots of the fraction of times female flies from the IV-M2 treatments associated with sub-chambers containing males of each body size class for each generation (indicated above each boxplot triplet). Horizontal lines represent the mean, with the box representing the 25th and 75th percentiles, the whiskers representing 1.5 times the interquartile range, and outliers represented by closed circles.

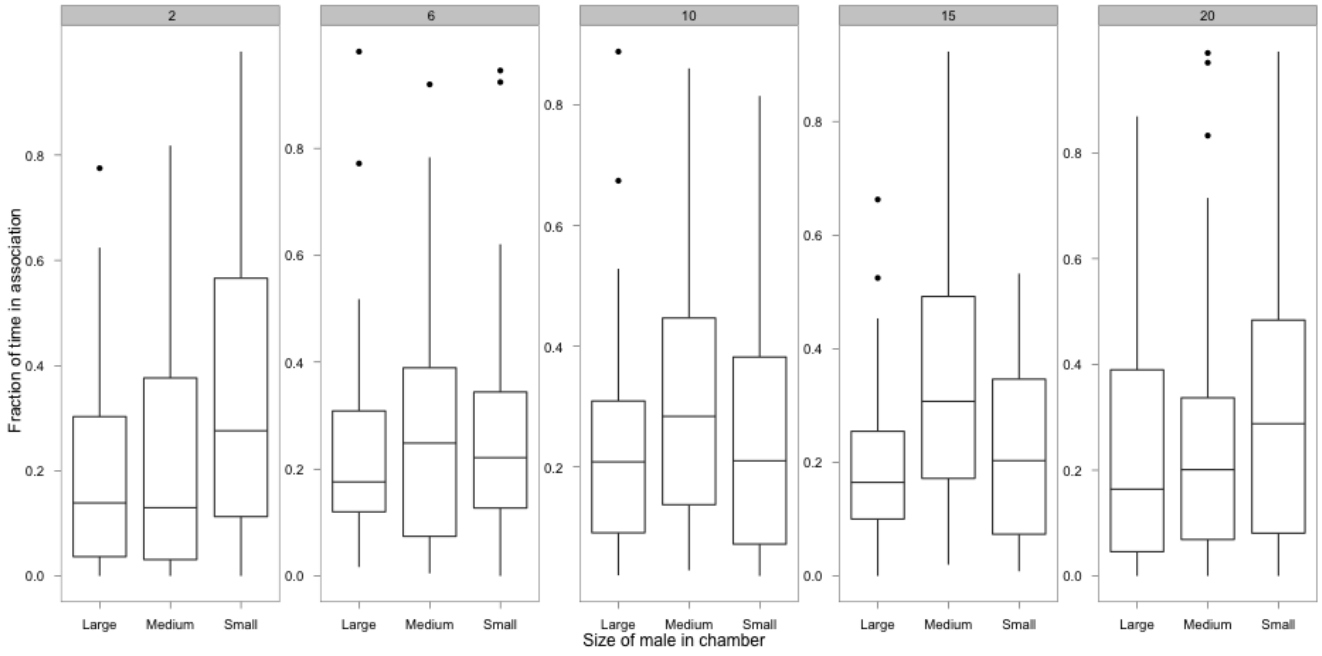

Figure 29. Boxplots of the fraction of times female flies from the LHm-L1 treatments associated with sub-chambers containing males of each body size class for each generation (indicated above each boxplot triplet). Horizontal lines represent the mean, with the box representing the 25th and 75th percentiles, the whiskers representing 1.5 times the interquartile range, and outliers represented by closed circles.

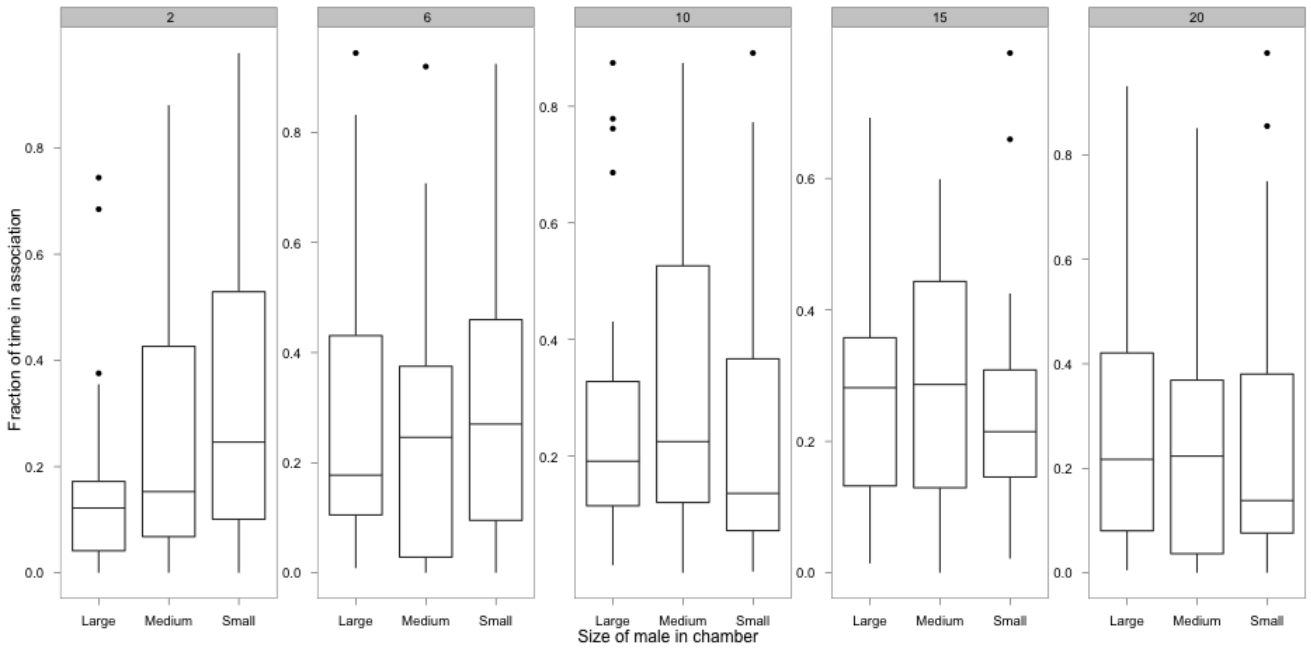

Figure 30. Boxplots of the fraction of times female flies from the LHm-L2 treatments associated with sub-chambers containing males of each body size class for each generation (indicated above each boxplot triplet). Horizontal lines represent the mean, with the box representing the 25th and 75th percentiles, the whiskers representing 1.5 times the interquartile range, and outliers represented by closed circles.

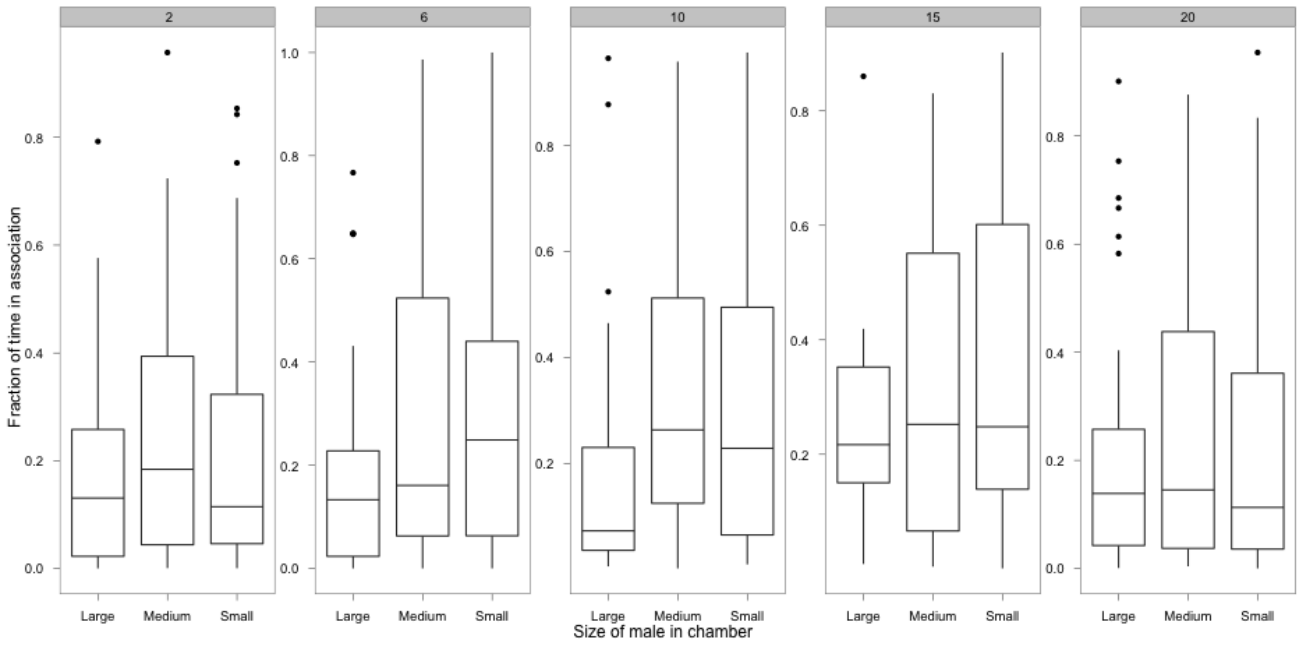

Figure 31. Boxplots of the fraction of times female flies from the IV-L1 treatments associated with sub-chambers containing males of each body size class for each generation (indicated above each boxplot triplet). Horizontal lines represent the mean, with the box representing the 25th and 75th percentiles, the whiskers representing 1.5 times the interquartile range, and outliers represented by closed circles.

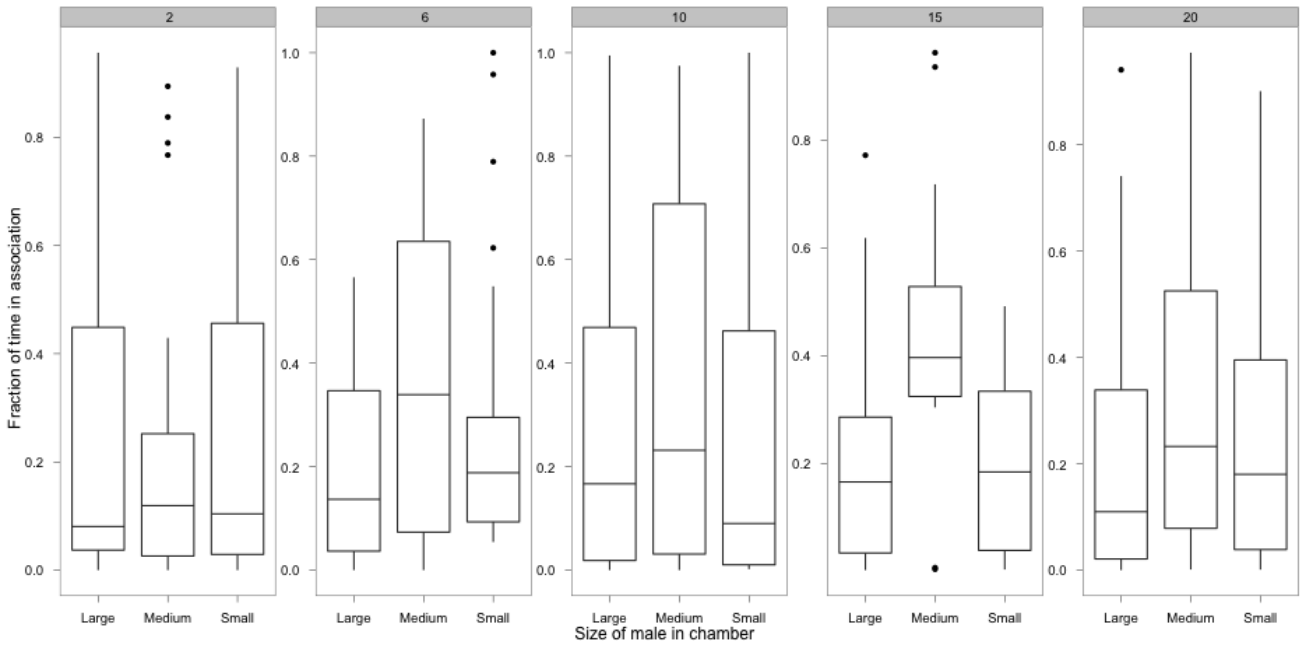

Figure 32. Boxplots of the fraction of times female flies from the IV-L2 treatments associated with sub-chambers containing males of each body size class for each generation (indicated above each boxplot triplet). Horizontal lines represent the mean, with the box representing the 25th and 75th percentiles, the whiskers representing 1.5 times the interquartile range, and outliers represented by closed circles.

Treatment	Estimate (slope)	t value	df	*p*
IV-L1 males	23.61	0.211	19	0.835
IV-L2 males	-17.5	-0.191	18	0.85
IV-M1 males	7.593	0.087	19	0.932
IV-M2 males	-196.3	-1.674	18	0.111
IV-S1 males	51.6	0.34	19	0.737
IV-S2 males	-50.31	-0.386	18	0.704
LHm-L1 males	-236	-1.587	19	0.129
LHm-L2 males	106.5	0.544	18	0.593
LHm-M1 males	97.03	0.511	19	0.616
LHm-M2 males	103	0.947	18	0.356
LHm-S1 males	-152.5	-1.375	19	0.185
LHm-S2 males	107.8	0.732	18	0.473

Table 3. Variance in male body size in each EE treatment fitted to a linear model over 20 generations.

Treatment	Estimate (slope)	t value	df	*p*
IV-L1 females	119.25	1.455	19	0.162
IV-L2 females	-75.16	-0.785	18	0.443
IV-M1 females	152.69	2.498	19	0.022
IV-M2 females	-98.16	-0.961	18	0.349
IV-S1 females	155.8	1.318	19	0.203
IV-S2 females	45.59	0.394	18	0.698
LHm-L1 females	-8.653	-0.062	19	0.951
LHm-L2 females	187.9	1.584	18	0.131
LHm-M1 females	103.3	0.632	19	0.535
LHm-M2 females	220.7	2.138	18	0.0465
LHm-S1 females	72.25	0.658	19	0.518
LHm-S2 females	244.3	1.691	18	0.108

Table 4. Variance in female body size in each EE treatment fitted to a linear model over 20 generations.

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4.0 General Discussion

Differences in reproductive success are what allow sexual selection to occur. While competition between members of one sex (intrasexual selection) is an important aspect of sex-specific trait evolution, selection imposed by choosiness by members of the opposite sex (intersexual selection) also greatly influences how traits evolve in a population. In my thesis I primarily examined aspects of this latter process in two different experiments where I controlled for intrasexual selection by eliminating male-male competition in behavioural assays.

Sexual size dimorphism exists in many species because of modifications in physiology or morphology required for sexual reproduction. One sex usually needs to invest more energy than the other in reproductive processes, such as producing gametes, rearing offspring, or providing parental care. Thus, for the sex that has a disproportionately larger energy requirement (often the "females" in many species), it is often necessary (and advantageous) to be physically larger in order to meet these extra energy demands. Furthermore, if the "quality" of one's mate is directly related to the potential fitness of potential offspring (indirect benefits) or the quality/amount of physical resources provided toward reproduction (direct benefits), it may be in a female's best interest to be highly discriminatory when choosing a mate (in order to maximize fitness). Many species have been observed to exercise mate choice based on body size; body size is usually a reliable indicator of individual condition in both sexes (Andersson, 1994). Consequently, this has made body size an attractive trait for experimental study, as environmental variation can be tightly controlled in a laboratory setting. Additionally, because of the ease with which populations can be monitored and manipulated in a

laboratory, gene flow between populations can be completely controlled and large population sizes can be maintained to reduce the likelihood of genetic drift.

Studies using *D. melanogaster*, a species possessing significant sexual size dimorphism, have previously reported mate choice based on body size (Ewing, 1961) and are thought to possess significant amounts additive genetic variation (Tantawy, 1957; Tantawy, 1959). While past studies have examined the role of body size in both sexes on aspects of mate choice (Partridge *et al.*, 1987; Pitnick, 1991; Pitnick and Garcia-Gonzalez, 2002; Byrne and Rice, 2006), only one study has quantified how body size variation in both sexes affects aspects of mate choice (Turiegano *et al.*, 2012). However, this study had several potential limitations, such as the fly stock used, culture protocol, and amount of actual body size variation present in the males and females. Thus, Chapter 2 of my thesis endeavored to determine how body size in both sexes influenced several pre and post-copulatory mating behaviours. I found that body size differences in male and female D. melanogaster significantly affected pre and post-copulatory mating behaviours. Furthermore, I found evidence that *D. melanogaster* may use relative body size differences, rather than absolute body size differences, in determining how long to wait before initiating courtship. Surprisingly, I failed to detect an effect of relative or absolute body size on copulation length, despite previous studies reporting a significant effect of absolute body size on this behaviour (Partridge *et al.*, 1987; Pitnick and Garcia-Gonzalez, 2002). Most studies examining copulation length have used flies that were derived from either isolines (Turiegano *et al*., 2012), highly inbred lines (Pitnick and Garcia-Gonzalez, 2002), or used lines that had had only a short time to adapt to the laboratory (Pitnick, 1991), which may partially explain why I did not detect a difference.

My second study (Chapter 3) revealed that female preference variation had a genetic component by using artificial selection that resulted in behavioural and phenotypic changes over multiple generations. I observed significant change in the frequency of brown-eyed dominant flies over the duration of the study, with both EE-IV and EE-LHm lines showing decreased numbers of brown-eyed dominant flies over time. I observed body size changes in each treatment compared to the generation 0 control (i.e. no artificial selection). "Large" body size treatments (i.e. selecting against female preference for large body sizes) resulted in smaller males and females in both EE-IV and EE-LHm lines. "Medium" body size treatments resulted in smaller males and females in both EE-IV and EE-LHm lines. "Small" body size treatments generally resulted in further decreases in the body size of both males and females in both EE-IV and EE-LHm lines (though I found significant body size increases for males in one EE-LHm replicate). Finally, I found significant differences in female sub-chamber associations (i.e. behavioural differences) in several generations: in "large" body size treatments, females from EE-IV lines showed differences in generation 2 and 20 in the predicted direction (i.e. association with larger males). In "medium" body size treatments, females from EE-IV lines showed differences in generations 6, 10, and 15 in the predicted direction (i.e. association with larger males). Lastly, in "small" body size treatments, females from EE-LHm lines showed differences in generations 2 and 15 in the predicted direction (i.e. association with smaller males). I am unaware of anyone who has done work that directly selected on female preference for male body size in *D. melanogaster*. The closest similar work done has used direct selection on female preference for male body size in guppies (*P. reticulata*) (Hall et al., 2004). Hall *et al*'s (2004) study failed to detect a response by

directly selecting for female preference for an attractive male trait, and on male attractiveness. Female *P. reticulata* demonstrate mate choice based on pigmentation, a sexually dimorphic trait, with brightly coloured males tending to be preferred by females (though not universally in across all populations (see Houde, 1988)). Male *P. reticulata* court females through a courtship dance, where they flex their bodies into an S shape and vibrate. The maintenance of this courtship behaviour, which requires strength against a current, has been correlated with the degree of pigmentation in males (Nicoletto, 1996). Thus, pigmentation serves as a primary indicator of fitness in *P. reticulata*. However, *D. melanogaster* demonstrate mate choice based on a wide array of phenotypic, morphological, and biochemical factors, such as body size (Spieth, 1952), eye colour (Ribó *et al*., 1989), sex comb number (Cook, 1977), cuticular hydrocarbon profile (Antony and Jallon, 1982), and wing vibration frequency during courtship (McDonald and Crossley, 1982). That I observed a response where Hall *et al.* (2004) did not may have been simply been due to differences in the study organism used, however the number of generations of artificial selection, effective population size, and method of artificial selection may also have significantly influenced the results of my experiments.

Both of my studies attempt to answer the question of how the outcomes of social interactions in *D. melanogaster* are influenced by variation in body size; specifically, how both male and female body size contribute to pre- and post-copulatory mating behaviours, and if variation present in females (that influence body size preference for males) has a genetic component. That both contribute toward furthering our understanding of how body size is related to mating behaviour will allow future studies using *D. melanogaster* to better interpret male and female interactions that involve

mating behaviours. Future work should continue investigating how female preference is influenced by male and female body size variation. In order to accomplish this, several approaches could be applied to future studies in order to provide a more comprehensive framework of how *D. melanogaster* respond to variation in body size. Firstly, a variety of *D. melanogaster* stocks should be used (including other large, outbred stocks) for investigating traits when genetic variation is known (or suspected) to exist. Past studies that have reported significant results have used stocks that were derived from isolines (i.e. lines that originated from a single inseminated female) (Turiegano *et al*., 2012), highly inbred lines (Pitnick and Garcia-Gonzalez, 2002), or lines that had only a short time to adapt to the laboratory environment and food source (Pitnick, 1991). While these stocks have utility in specific areas of investigation, they are not useful in the context of investigating female preference because of their limited genetic variation (in the case of isolines and inbred lines) or potential confounding effects generated by natural and sexual selection in a new environment (in the case of newly formed laboratory populations) (Sgro and Partridge, 2000; Hoffmann *et al.* 2001; Orozco-terWengel *et al*. 2012). Secondly, wide phenotypic ranges of body size should be generated using methods other than larval crowding. While this approach succeeds in decreasing the mean body size of flies (Alpatov, 1932), it also affects nearly all components of fitness (Ribó *et al*., 1989). It is therefore conceivable that studies which utilized larval crowding for generating body size variation may have introduced significant confounding effects into their measurements of various mating behaviours. Finally, statistical analyses should account for both absolute and relative size differences between individuals. Given that my first study found a significant difference for time to courtship initiation when using a relative

body size comparison, compared to detecting a significant difference for length of courtship when using an absolute body size comparison illustrates how individual flies may not always perceive body size in terms of absolute differences.

In this thesis, I have used individual assays to investigate multiple facets of mating behaviour. In my first study, I considered behavioural differences between individual flies and correlated those differences with body size in both sexes. In my second study, I not only considered behavioural differences between individual flies, but also considered population-wide behavioural differences by using two different stocks of wild-type flies. Furthermore, by imposing selection against female preference, changes at the genetic level manifested as behavioural differences related to body size. My integrative biology thesis incorporate aspects of individual, within-population, and between-population differences into a comprehensive framework and links results from across multiple fields of biology. Together, these two works make this thesis an important contribution to the field of integrative biology.

4.1 References

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