

Dispersal-reproduction trade-offs in *Drosophila*: implications for geographic distributions

by

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Declaration

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Summary

Life-history and performance trade-offs are common among insects. One major trade-off reported and frequently studied in a handful of taxa (e.g. butterflies, moths and crickets) is a dispersal-reproduction trade-off, such that individuals within a species that choose to disperse typically sacrifice reproductive output. However, the generality of this hypothesis has not been well examined, and its implications for invasion biology, geographic distributions and responses to climate change have yet to be fully determined. Here, I aimed to experimentally measure the magnitude and direction of potential dispersal-reproduction trade-offs between flies that choose to disperse (dispersers) and those that do not (resident) from five *Drosophila* species collected within South Africa, varying in their ecology. Next, to better understand how flight performance may be correlated to geographic range extent and potential responses to climate variability, I estimated the thermal performance curves of flight ability in 11 *Drosophilidae* species after two generations under standard laboratory conditions, and tested if any major morphological or ecological factors (e.g. sex, body mass, wing loading, geographic range size) predicted traits of thermal flight performance curves (optimum temperature, maximum performance, breadth of performance). My results showed two major sets of findings. First, there was only weak support for the dispersal-reproduction trade-off and that the reproduction trait scored (egg number vs. adult survival) yields different outcomes. For reproductive fitness (offspring's adult emergence rates), only one of the five species (*Drosophila melanogaster*) tested showed statistically significant evidence of the trade-off in the direction expected. Furthermore, there was no correlation between the magnitude of the trade-off and geographic distribution ranking. Second, aspects of thermal performance curves for flight differed between species; however, the best explanatory model of these flight responses included significant positive effects of test temperature and wing area. Rank of the breadth of geographic distribution and phylogeny failed to explain significant variation in any of the traits of thermal flight performance curves in these 11 species. In conclusion, I argue that dispersal-reproduction trade-offs are unlikely to contribute greatly to an insect species' invasion success or its geographic distribution, although environmental temperature and wing size will mediate dispersal responses upon introduction into novel environments. This study therefore suggests that dispersal-reproduction trade-offs are not as common as might have been expected when assessed systematically, at least not in the genus *Drosophila*, and moreover, that the extent of such trade-offs, and their magnitude and direction, require further investigation.

Opsomming

Kompromieë in lewensgeskiedenis en prestasie is algemeen onder insekte. Een belangrike kompromie wat dikwels berig word en gereeld bestudeer word in 'n handjievol taksa (bv. skoenlappers, motte en krieke) is 'n verspreiding-voortplantingskompromie, waarvolgens individue van 'n spesie wat kies om te versprei hulle reprodutiewe uitsette prysgee. Die algemene toespasbaarheid van hierdie hipotese is egter nog nie goed ondersoek nie en die implikasies daarvan vir indringerbiologie, geografiese verspreiding en reaksies op klimaatsverandering is nog nie ten volle bepaal nie. Hier het ek dus eksperimenteel die omvang en rigting van potensiële verspreiding-voortplantingskompromieë getoets tussen vlieë wat versprei (verspreider) en diegene wat nie versprei nie (inwoner) in vyf *Drosophila*-spesies met variasie in hul ekologie wat in Suid-Afrika versamel is. Voorts, om te bepaal of vlugprestasie korreleer met geografiese verspreiding sowel as potensiële reaksies op klimaatsveranderlikheid, het ek die termiese-prestasiekurwes van vlugvermoë in 11 *Drosophilidae*-spesies bepaal na twee generasies onder standaard laboratoriumkondisies. Ek het ook getoets of enige morfologiese of ekologiese faktore (bv. geslag, liggaamsmassa, vlerk-lading, geografieseverspreiding) belangrik is om die termiese vlug prestasie kurwes (optimum temperatuur, maksimum prestasie, breedte van prestasie) te beskryf. My resultate het twee belangrike uitkomstegedhad. Eerstens, was daar slegs geringe ondersteuning vir die verspreiding-voortplantingskompromie en die uitkomste het verskil afhangende van die voortplantingskenmerk wat getoets is (getal eiers teenoor volwasse oorlewing). Vir reprodutiewe fiksheid (aantal volwasse nageslag), het slegs een van die vyf spesies (*Drosophila melanogaster*) statisties beduidende ondersteuning getoon vir die verspreiding-voortplantingskompromie. Daar was ook geen verband tussen die omvang van die kompromie en die rangorde van die geografiese verspreiding nie. Tweedens, aspekte van termiese-prestasiekurwes vir vlug het verskil tussen spesies en die beste verklarende model het beduidende positiewe effekte van toetstemperatuur en vlerkarea ingesluit. Rangorde van die breedte van geografieseverspreiding en filogenie het versuim om betekenisvolle variasie in enige van die eienskappe van termiese-prestasiekurwes in die 11 spesies te verduidelik. Ten slotte voer ek aan dat 'n verspreiding-voortplantingskompromie waarskynlik nie bydra tot die sukses van 'n insekspesies se verspreidings sukses of geografiese verspreiding nie, alhoewel omgewingstemperatuur en vlerkgrootte verspreiding in nuwe omgewings kan bevorder. Hierdie studie dui dus daarop dat verspreiding-voortplantingskompromieë nie so algemeen voorkom as wat verwag sou word wanneer dit sistematies geëvalueer word nie, aldus nie in die genus *Drosophila* nie. Die omvang en rigting van hierdie kompromieë vereis verdere ondersoek.

This thesis is dedicated to the memory of my avô, Antonio De Araujo.

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Preface

This thesis comprises of a single chapter.

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1. Introduction

An invasive organism is one that has been introduced to habitats outside of their native range and that has established a permanent population. Typically, this invasion process is aided by global anthropogenic movement (Hulme, 2009; Wilson et al., 2009). Invasive species pose significant threats to native biota (IUCN, 2000) and a diverse range of ecosystem services and, therefore, may have far-reaching impacts on human and animal health or food security. Biological invasions have shown to be an important driving force influencing native biodiversity, enhancing habitat degradation and changing ecosystem functions (Troost, 2010; Wagner and Van Driesche, 2010). The success of an invasive species typically cannot be determined by a single characteristic of the organism or habitat being invaded but rather a combination of ecological and biological characteristics (Devin and Beisel, 2007; Hill et al., 2016). Characteristics of an organism that contribute to a successful invasion include a larger body size, high fecundity and long lifespan (Newsome and Noble, 1986; Bij de Vaate et al., 2002; Schneider, 2008). On the other hand, ecological or environmental factors that contribute to the invasibility of a habitat include the abundance of niche resources and an absence of natural enemies ('enemy release' hypothesis) (Roy et al., 2002; Colautti et al., 2006; Richards et al., 2006; Burton et al., 2010; Kajita and Evans, 2010). However, the number and frequency of introduced individuals, termed propagule pressure, is often a major factor determining the establishment of invasive species outside their native range rather than the aforementioned characteristics (Lockwood et al., 2005; Colautti et al., 2006; Lockwood et al., 2009; Simberloff, 2009; Wittmann et al., 2014; Cassey et al., 2018).

The successful establishment of an introduced species has been shown to link with propagule pressure; the more individuals introduced, the higher the establishment success rate (Memmott et al., 1998; Lockwood et al., 2009; Blackburn et al., 2013). However, in some cases the physical attributes of the invading organism, such as performance or behavioural traits, as well as the absolute quantity of invaders influences invasion successes (Lange and Marshall, 2016). Propagule pressure has proved particularly significant for establishment in biological control agent introduction success (e.g. Grevstad, 1999; Duncan et al., 2014). Higher numbers of introductions or a greater number of individuals (in both cases termed higher propagule pressure) result in a better chance of more individuals establishing and increasing the population numbers over time. Propagule pressure plays a key role in invasion success as it allows species to overcome environmental stochasticity and/or other stressors, including Allee effects (Von Holle and Simberloff, 2005; Simberloff, 2009; Duncan et al., 2014) which allows biological and ecological characteristics to contribute to range expansion post-

establishment. Together with frequent introductions, also comes increased genetic diversity, which can eliminate or at least lessen the severity of the expected population bottleneck post-introduction, thereby leading to an increased chance of survival of the introduced species (Simberloff, 2009; Bertelsmeier and Keller, 2018).

For an invasion to take place, a non-native species needs to spread through some form of dispersal and expand across geographic boundaries or breaks in suitable habitat (Wilson et al., 2009). Dispersal ecology is complex and well-reviewed (e.g. Bonte et al., 2003, 2010, 2012), with a host of interacting factors determining a species dispersal potential (e.g. Esterhuizen et al., 2014; Steyn et al., 2016), and another host of factors influencing the realized outcomes of different forms of dispersal. However, the extent to which fine-scale population dynamics and local dispersal (e.g. an individual's home range size or daily movement patterns) translates to broad-scale species' geographic distributions is the subject of long interest in the fields of ecology and evolution, often studied under the broader theme of biogeography (Gaston, 2003), macroecology (Gaston et al., 2009) or metapopulation dynamics (Hanski, 1998). Ultimately, long-distance dispersal is important particularly in invasive species as it allows them to reach new areas by overcoming profound geographical barriers that would otherwise not have been possible (Kot et al., 1996; Parendes and Jones, 2000). The opportunity for dispersal across geographic barriers has become easier for species with increased anthropogenic movement (Wilson et al., 2009). Once a species has successfully crossed such a geographic barrier, often with human assistance (e.g. Karsten et al., 2015), and establishes a population in the new habitat, fine-scale dispersal comes into play and influences rates of spread through intrinsic (more routine and regular) movement patterns. The latter type of fine-scale, daily spread dynamics, and how these might interact with patterns of local population dynamics and the environment, are the main subject of this thesis and the research presented here, while acknowledging that these movement patterns at fine spatial and temporal scales can result in contributing to broader patterns of species' geographic distributions.

Dispersal is widely acknowledged as being crucial for the ecology and evolution of organisms in terrestrial and marine environments so as to spread genes, avoid intraspecific competition, locate food and mates and to escape threats such as habitat disturbances and fragmentation or predators (Culik, 2001; Langellotto et al., 2001; Trakhtenbrot et al., 2005; Anderson et al., 2008; Bonte and Van Dyck, 2009). However, dispersal typically requires time and energy investment (Bonte et al., 2012), and can affect performance and survival through trade-offs among key traits (Baker and Rao, 2004). Such costs or trade-offs, summarised by Bonte et al. (2012), include survival, opportunity and time costs in a new area due to unfamiliarity (Part, 1991; Brown et al., 2008), lifestyle-specific traits (e.g. nictation in

ambush foraging nematodes) (Bal et al., 2014), energy trade-offs (Nespolo et al., 2008), minor energy costs (Vahl and Clausen, 1980; Basson et al., 2017), and, important for this study, reduced reproductive ability (Karlsson and Johansson, 2008). Reproductive ability, however, may refer to a diverse array of metrics of reproductive effort, success and failure, which includes for example, egg size and number, egg-to-adult viability or egg hatching success rates. These may be more (or less) related to evolutionary fitness, although sometimes it is unclear of the best or most relevant metric by which to measure such trade-offs (Zera and Brink, 2000; Zhao and Zera, 2001; Zera and Zhao, 2006; Zhao and Zera, 2006).

Fitness consequences of dispersing have been shown in a variety of different traits. Longevity, for example, has a negative correlation with dispersal (Gu et al., 2006; Khuhro et al., 2014). Stevens et al. (2012) emphasises the importance of wing size for increasing dispersal distance since larger wing size, or lower wing loading (calculated as body mass/wing area), allows for great dispersal propensity in different butterfly species (reviewed in Dudley, 2002). However, Dillon and Frazier (2006) found wing loading to have no significant influence on flight performance in *D. melanogaster* under varying humidity and temperature. Differences in wing morphology between sexes are frequently reported, and often consistent with the direction of dispersal ability variation. For example, the morphological differences frequently reflect that males have enhanced flight performance, perhaps for foraging or locating a mate, while females typically show morphologies associated with their increased reproductive effort (e.g. egg load) and therefore reduced dispersal ability (Stevens et al., 2012).

Ambient temperature affects many traits in ectotherms including for example metabolism, growth rate and locomotor performance (Gillooly et al., 2001, Ashton, 2004; Dillon and Frazier, 2006; Angilletta, 2009; Clusella-Trullas et al., 2010). Thermal performance curves (TPCs) have been argued to be able to help forecast wholesale species range shifts (Buckley et al., 2012; Buckley et al., 2015; Buckley and Huey, 2016) in response to predicted climate change and also changes in performance (e.g. population feeding or growth rates) with more subtle changes in environmental conditions (Angert et al., 2011; Dell et al., 2011; Huey and Kingsolver, 2011; Barton & Terblanche, 2014; Sinclair et al., 2016). The influence of ambient temperature on flight responses or relative performance has been researched extensively across diverse insect taxa (Dillon and Frazier, 2006; Karlsson and Johansson, 2008; Zhang et al., 2008; Samejima and Tsubaki, 2010; Esterhuizen et al., 2014; Mason, 2017). For example, most fly species examined to date will fly more readily at higher temperatures, although prior thermal history may alter the nature of this response (*Drosophila melanogaster* in Dillon and Frazier, 2006; Frazier et al., 2008; c.f. *Ceratitis capitata* in Esterhuizen et al., 2014). Therefore, thermal flight performance curves (TFPCs) may be a useful proxy for forecasting local and regional changes in

pterygote (i.e. winged) insect species, such as species within the Diptera (e.g. Drosophilidae). To my knowledge, however, surprisingly few studies have assessed TFPCs across a range of species under standard laboratory conditions despite that it may be useful in predicting performance or range shifts with the forecasted climate patterns and given the group's value in understanding such climate change responses (e.g. Overgaard et al., 2014; Bush et al., 2016; Kellermann et al., 2018; reviewed in Hoffmann, 2010).

The reproduction-dispersal trade-off is the occurrence of decreased fecundity due to increased distance travelled (Figure 1) and has been investigated across different insects such as butterflies (Hughes et al., 2003; Karlsson and Johansson, 2008), crickets (Roff, 1984; Stirling et al., 2001) and moths (Gu et al., 2006). Most research focuses on wing-dimorphic species (Guerra, 2011). Mole and Zera (1993), for example, investigated the sacrifice of reproductive efficiency in long-winged female crickets by allocating energy to flight muscles and larger wings rather than into egg production like their short-winged counterparts. Some research has focused on wing monomorphic species such as beetles (David et al., 2015) and moths (Gu et al., 2006) but there is limited research for varying taxa. With increasing dispersal and expanding species' distributions forecasted due to climate change (Pecl et al. 2016), species population numbers could be altered (Hughes et al., 2003). This could aid successful invasions for species that overcome this trade-off or are not penalised heavily for enhanced dispersal. This trade-off has been shown to be absent, or at least less evident, in some invasive plant species (Lambrecht-McDowell and Radosевич, 2005) but pronounced in the native population of an invasive aphid (Zhang et al., 2008). There is insufficient data however, on the magnitude and extent of such a trade-off in other invasive or native insects. Moreover, in cases where such a trade-off exists, the duration of persistence of the trade-off across generations typically remains unclear (Khuhro et al., 2014) but is significant for understanding evolutionary responses.

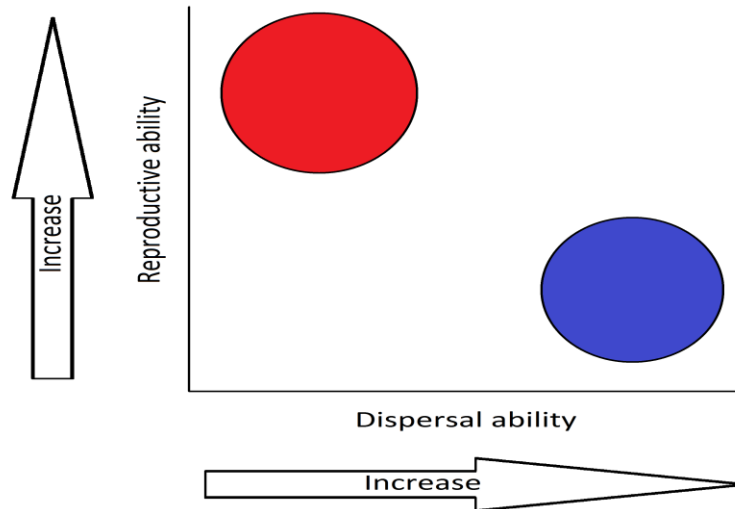


Figure 1: A graphical model illustration of the dispersal-reproduction trade-off hypothesis whereby high dispersal ability results in low reproductive ability (blue circle) and high reproductive ability is associated with low dispersal ability (red circle).

To investigate dispersal ability over a broad temperature range, and how dispersal-reproduction trade-offs might manifest with geographic distribution or invasion success, I chose to study this question in Drosophilidae as a model system for at least four main reasons. First, knowledge of South African species diversity and geographic range extent or population abundances in this group is poor (McEvey et al., 1988), thus limiting the group's utility to address key climate, evolutionary or ecological questions in an African context. Second, flies in the Drosophilidae could serve as excellent models to study dispersal ecology and invasion biology (Gibert et al., 2016). For example, *D. melanogaster* has been used as a model system for multiple human-related diseases (various pathologies and syndromes) and can replace ethically-controversial mammal models, owing largely to a significant overlap in biochemical pathways and genetic similarity between *Drosophila* and human physiology (Potter et al., 2000; Bier and Bodmer, 2004; Wolf et al., 2005; Pandey and Nichols, 2011; Hewitt and Whitworth, 2016; Sonoshita and Cagan, 2016). Third, drosophilids are not only good model species' because of abundant information available for most species, such as their phylogenetic information (O'Grady and DeSalle, 2018) but also their ecology and geographic distributions are increasingly well documented in various/select regions (Carareto, 2011; Bächli, 2018). Many drosophilid species have wide (cosmopolitan) distributions yet there are also important specialist and/or narrow range endemic species (Kellermann et al., 2009), with some key species expanding their range readily (e.g. *Drosophila suzukii*) (Vilela and Goñi, 2015; Gibert et al., 2016), although there is typically limited data covering Drosophilidae species' historical native distributions. Finally, Drosophilidae continue to be an

important model for testing climate change responses of terrestrial insects and understanding evolutionary constraints and adaptive capacity of animals more broadly (e.g. Kellermann et al., 2009; van Heerwarden and Sgrò, 2014; Bush et al., 2016).

Many factors need to be considered when attempting to understand the ability of a species or population to occupy their specific geographic distribution or range extent (Lester et al., 2007; Elith and Leathwick, 2009; Gaston, 2009, Elith et al., 2010; Kearney et al., 2010; Chown, 2012). With almost 4500 Drosophilidae species described to date (Bächli, 2018), most drosophilid species have been disregarded as agricultural pests as they prefer over-ripe and rotting fruit (Hodge, 1996). Some drosophilid species have invaded various parts of the world (Nicolson, 1994; Gibert et al., 2010; Deprá et al., 2014), most notably, *D. suzukii* and *Zaprionus indianus*. The latter are well-known species of the Drosophilidae family that have become pests to ripe soft-skinned fruits and are considered to be global invasive species (Calabria et al., 2012). Berry and grape farms, in e.g. USA and south-western Europe, have since invested a lot of effort and resources into managing these pests (Cini et al., 2014; 2014; Grant and Sial, 2016; Farnsworth et al., 2017). Other invasive drosophilid species includes *D. subobscura* in the Americas (Pascual et al., 2007) which is not considered a pest but the implications on native species is unknown. With many drosophilids having wide geographic ranges, these species have great potential to expand their niches (Hill et al., 2017) and thus likely also an ability to readily invade new areas. However, remarkably little information is available regarding Drosophilidae species in South Africa (McEvey et al., 1988), with the most recent records showing 76 species in the country documented between 1900 and 2013 (McEvey, 2016). More recent records include *D. simulans* (2013), *D. punctatonervosa* (2013), *Scaptomyza oxyphallus* (2013) and *D. immigrans* (2010). Although *D. flavohirta* (1983) has been recorded as an invasive drosophilid in South Africa, no recent information regarding this species or their impacts are available.

Here, I therefore aimed to experimentally measure the magnitude and direction of dispersal-reproduction trade-offs between flies that choose to disperse (dispersers) and those that don't (residents) from five *Drosophila* species collected within South Africa varying in their ecology and likely physiological performances. Next, to better understand how flight performance may be correlated with geographic range extent (i.e., distribution) and potential responses to climate variability, I estimated the TFPCs of flight ability in 11 Drosophilidae species after they completed two generations under laboratory conditions and tested if any major morphological or ecological factors (e.g. sex, body size, wing loading, geographic range size) predicted the main parameters (e.g. optimum temperature, maximum performance, breadth of performance) of species-level TFPCs. These results are discussed

in the context of insect invasions, geographic ranges, and potential climate change responses. I predict that dispersers with narrow distributions will have lower reproductive outputs than residents while there will be less or no difference for widely-distributed species. It is expected that flies with larger wings or lower wing loading will be better fliers. Species are expected to generally differ in their thermal flight response and morphology and this might be a consequence of shared evolutionary history (phylogenetic association).

2. Methods

2.1. Sampling and rearing

Drosophilidae (Figure 2) were sampled from six locations in the Western Cape and KwaZulu-Natal Provinces of South Africa (Table 1). The trapping method was adapted from Medeiros and Klaczko (1999) and consisted of bucket traps filled with ripe fruit (mango, grapes, lemon, oranges, figs, berries and bananas) or mushrooms purchased from local supermarkets in different combinations to capture as much Drosophilidae diversity as possible. Flies were collected by the placement of a plastic freezer bag over the bucket, touching the rims to ensure a complete seal and then shaken to probe the flies, causing them to fly up into the bag. The bags were then knotted, transported back to the laboratory where females were aspirated out and placed into plastic bottles filled with a prepared Bloomington's standard cornmeal diet medium (http://flystocks.bio.indiana.edu/Fly_Work/media-recipes/bloomfood.htm) to start iso-female lines. Species were identified using the Universal cytochrome oxidase I (COI) primers (Folmer et al., 1994) by Inqaba Biotech™ and the South African Sugarcane Research Institute's (SASRI) biotechnology department. Sequences were used to obtain species identifications by making use of the BLAST tool on the NCBI GenBank database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Eleven species were positively identified using the COI primer and three species were also identified by a drosophilid expert, Dr Shane McEvey (Australian Museum Research Institute) but only a sub-set of five of these 11 species could be successfully reared to the high numbers needed within the short time-frame of this project.

Iso-female lines were initiated from wild caught females. Flies captured in the Western Cape Province were reared at 23°C (Dillon and Frazier, 2006) on a 12:12 light dark cycle in a MRC LE-509 incubator (Holon, Israel) at the Applied Physiological Ecology Lab (Stellenbosch University) and those captured in KwaZulu-Natal at c. 25°C on a 12:12 light dark cycle in a temperature-controlled room at the South African Sugar Research Institute (SASRI). The rearing temperature in KwaZulu-Natal was slightly warmer, to better match the warmer, tropical climate of the region (Edward et al., 2010; Bretman et al., 2013). Once flies started emerging (F1) they were transferred to a new bottle with fresh diet. This process was replicated until the F3 generation for all species provide sufficient fly numbers for experiments and at the same time reduce/limit the effects of inbreeding, except one (*D. melanogaster*) for which the F2 generation was used due to time constraints. All experiments were conducted using six-day old flies only to try to avoid introducing variation associated with aging and senescence, which are well known in some traits from many insects including Drosophilidae (e.g. Bowler and Terblanche, 2008; Le Bourg, 2011; Colinet et al. 2015).

2.2. Dispersal-reproduction trade-off

To test the dispersal ability of the five *Drosophila* species successfully reared (Table 1), a 20m long tunnel (Figure 3) was constructed outdoors in full sunlight. In previous field experiments testing the effects of dispersal on diverse phenotypes, distances ranging between 2m and 30m have been used (Hoffmann et al., 2007; Kristensen et al., 2008; Overgaard et al., 2010) and I therefore considered 20m distance, and the considerable time it took to travel the full length of the tunnel (up to four hours in some species) sufficient to generate a trade-off, should one exist. The tunnel consisted of a structure made of 1m and 0.5m long 12mm diameter steel poles connected by welded steel joints covered by a fine mesh material cover made of curtain lining with a plastic window (40x30cm) on both ends and 'socks' (15cm diameter) at various points along the tunnel for easy access. Material dividers were sown on at various points along the length of the tunnel (2m, 10m, 18m) that could be closed, if desired, to prevent unwanted dispersal. A bottle filled with a random subset of 100 six-day-old virgin females (with even distribution from each iso-female line) were placed at one end of the tunnel and a fruit bait bucket (bananas, mangos, oranges and mushrooms) covered with mesh (to prevent flies from entering) were placed at the other. Experiments were restricted to sunny days starting at sunrise with maximum daily temperature above 24 °C due to poor responses at lower temperatures and wind speeds below 5m.s⁻¹. For each species, experiments were replicated three times with a single replicate per day. At the start of the experiment, the bottle was opened and shaken to ensure that all flies left the bottle. Flies were then allowed to disperse freely in the tunnel. When flies reached the bait-end of the tunnel they were aspirated out and placed in a vial with a single virgin male to mate. This was continued until 30 female dispersers were collected at the bait end of the tunnel and then the dividers in the tunnel were raised to prevent further dispersal. A random selection of 30 females that chose not to disperse were aspirated out of the 2m starting segment of the tunnel and placed in a vial with a virgin male to mate. The females that chose to fly to the end of the tunnel represent 'dispersers' and those that chose not to fly represent 'residents' and these terms will be used when referring to the dispersal-reproduction trade-off.

The vials were then transported back to the laboratory where each pair was placed inside a honey jar with three medium-filled bottle caps as oviposition sites and checked at 20-hour intervals until eggs have been laid (as a measure of fecundity). Once eggs were observed the males were removed and discarded; and the females were transferred to new vials with medium. The eggs were counted under a light microscope (Stemi 305, Zeiss, Germany) to measure reproductive effort. Female survival was monitored every day to determine longevity. Bottle caps with eggs were placed in a medium-filled

bottle and left to allow flies to complete their development and emerging adults were counted as a measure of reproductive fitness.

2.3. Thermal flight responses

Sixty six-day old male and female drosophilids, 15 of each sex for each test temperature from the 3rd generation (except in the case of *D. melanogaster* where 2nd generation flies were used) were used to test the thermal flight response in 11 species (Table 1; Figure 3). Performance was determined at four different controlled temperatures (16°C, 20°C, 24°C and 28°C) using a peltier plate cooler (CP-121, TE Technology, USA) and a temperature controller (TC-720, TE Technology, USA). In each instance, 15 males and females were tested and temperatures experienced by the flies monitored using a thermocouple (36 standard wire gauge, Type T, Omega, USA) connected to a hand-held thermometer (Fluke 52 II, Netherlands). A gauze swab was placed on the surface to prevent direct contact with the steel stage surface and a 20ml plastic container placed upside down on the gauze with the thermocouple inserted through a small hole melted on the side.

Flies were individually placed inside the container at the desired temperature and prodded a maximum of five times to minimize a potential bias introduced by only assessing flies that were enthusiastic or predisposed to take flight using a thin wire which resulted in either flight or no flight and were recorded as 1 and 0 respectively. 'Flight' (1) was defined as sustained flight across the container at least once with noticeable wing movements; 'no flight' (0) was recorded when the fly only jumped, walked or became immobile. Each fly was weighed (mg) after the experiment using a Mettler UMX2 microbalance scale (CH-8606 Greifensee, Mettler-Toledo GmbH, Laboratory & Weighing Technologies, Switzerland) and their wing surface area (mm²) determined. This was done by taking photographs using a Leica MZ16A auto montage microscope attached to a Leica DFC 290 fixed digital camera (Leica, Wetzlar, Germany); the wing perimeter manually outlined in ImageJ (<https://imagej.nih.gov/ij/>) which then calculated the area according to the scale unit of measurements provided to the software (Figure 4). The right wing was the preference for measurement but in instances where this wing had imperfections, the left wing was used. These measurements were considered and included here as they have previously been proven to influence flight performance in a diverse range of insect species (Harrison and Roberts, 2000; Berwaerts et al., 2002; reviewed in Dudley, 2002; Harrison et al., 2012).

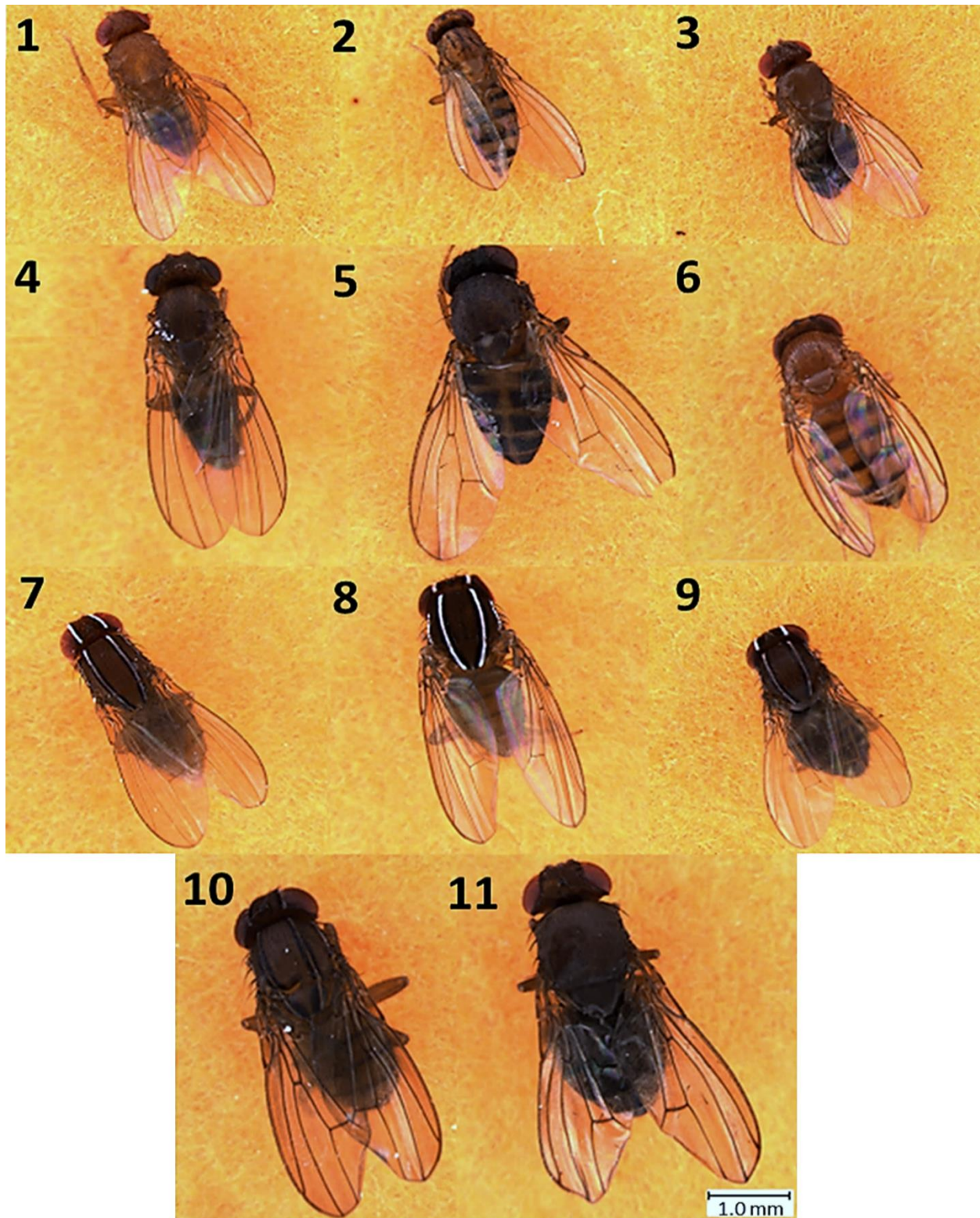


Figure 2: The 11 Drosophilidae species assessed in the thermal flight performance curve (TFPC) component of this study. 1) *Drosophila ananassae*, female 2) *D. busckii*, female; 3) *D. melanogaster*, female 4) *D. funebris*, male; 5) *D. hydei*, male; 6) *D. simulans*, female; 7) *Zaprionus indianus*, female; 8) *Z. taronus*, male; 9) *Z. tuberculatus*, male; 10) *Z. vittiger*, male; 11) *D. immigrans*, female. Note all species are represented at a shared scale of 1 mm.

Table 1: The locations of collection sites, date of capture, method of identification (Mol) as well as bait type for the 11 Drosophilidae species used in this study. All species were used in the thermal flight performance curve experiments (TFPC) and species used in the dispersal-reproduction trade-off experiment is indicated by the term 'both' (Exp group). The Rank indicates each species' geographic distribution assigned from widely spread (11) to narrowly spread (1). An asterisk (*) indicates which species have not previously been recorded in South Africa. The latitude and longitude is expressed in decimal degrees.

Species	Rank	Bait	Exp group	Latitude (°S)	Longitude (°E)	Date of Capture	Method of Identification
<i>Drosophila ananassae</i> *	4	orange and lemon mix	both	-29.7062	31.0444	22 March 2018	Sequence
<i>Drosophila busckii</i>	7	avocado	both	-33.8956	18.5642	2 November 2017	Sequence
<i>Drosophila immigrans</i>	9	banana	both	-33.8956	18.5642	2 November 2017	Sequence, expert
<i>Drosophila melanogaster</i>	11	banana, orange	both	-29.7013	31.1009	8 June 2018	Sequence
<i>Drosophila simulans</i>	10	orange	both	-33.8956	18.5642	2 November 2017	Sequence, expert
<i>Drosophila funebris</i>	5	banana	TFPC	-33.3465	19.623165	9 January 2018	Sequence
<i>Drosophila hydei</i>	8	mushroom, banana, orange	TFPC	-33.8956	18.5642	11 October 2017	Sequence, expert
<i>Zaprionus indianus</i>	6	banana	TFPC	-29.7062	31.0444	22 March 2018	Sequence
<i>Zaprionus taronus</i> *	2	lemon	TFPC	-33.9353	18.8907	16 November 2017	Sequence
<i>Zaprionus tuberculatus</i>	1	banana	TFPC	-29.7062	31.0444	22 May 2018	Sequence
<i>Zaprionus vittiger</i>	3	mango	TFPC	-33.9365	18.8657	20 October 2017	Sequence

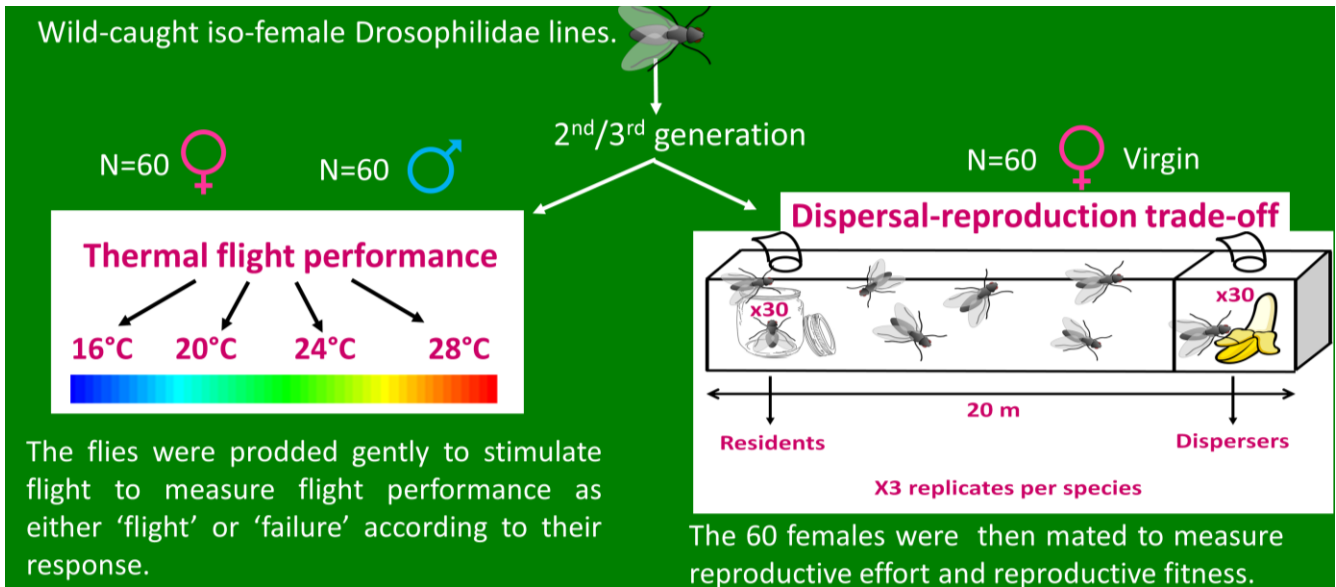


Figure 3: A graphic representation of thermal flight performance methods (left) and reproduction-dispersal trade-offs (right). Please see main text for details.

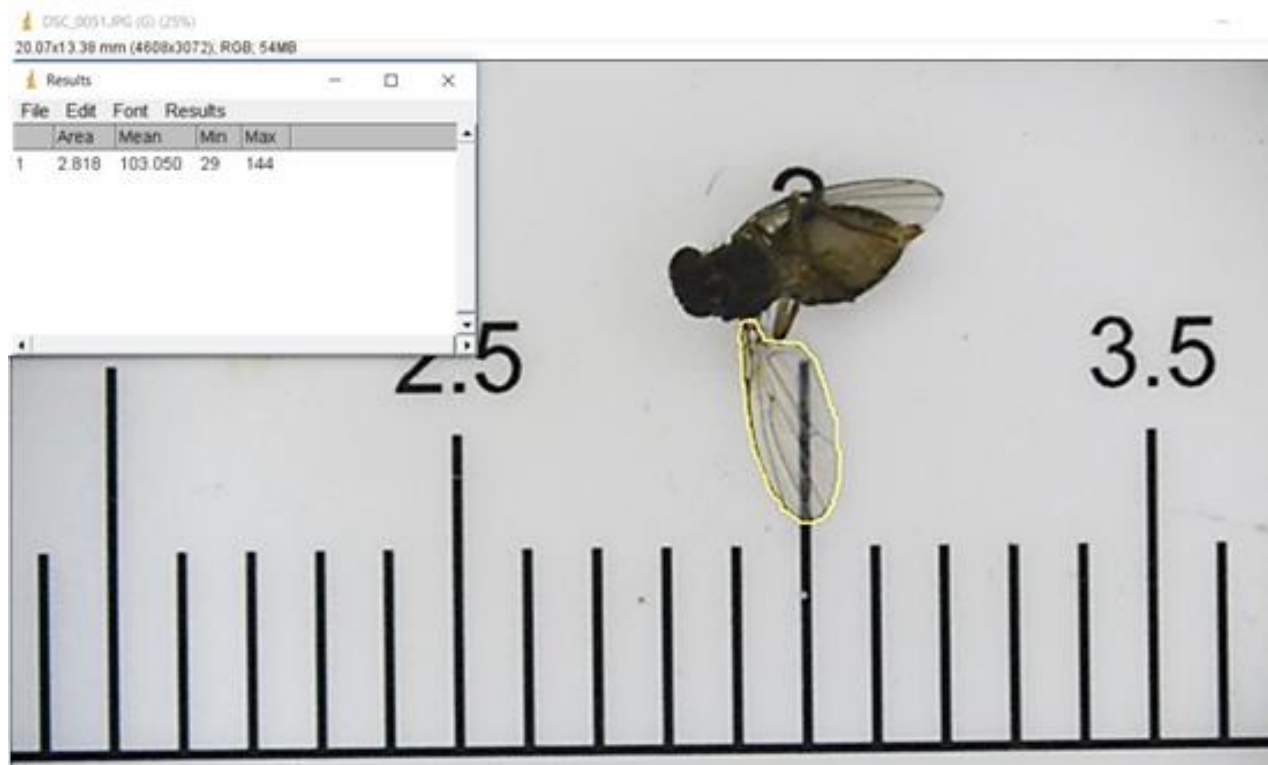


Figure 4: An example of the process of measuring the wing area of a single *Drosophila* specimen using ImageJ. To calculate the wing area, the scale was set using a ruler in mm and the wing outline traced manually. ImageJ provides the calculated area of the outlined object in mm².

2.4. Statistical analyses

Species were ranked according to their relative geographic distribution with the highest number (11) being the most widely distributed and the lowest number (1) having the most narrow distribution or more specialised distribution and will be referred to as 'rank' in the rest of this thesis. The ranking was assigned according to the distributions available on TaxoDros (www.taxodros.uzh.ch/search/dist_reg.php). Species distributed across different habitat types were classified as more widely-distributed and assigned a higher value and the assigned ranking value decreased as species ranges became more restricted or specialised (for example, those only found along the equatorial belt or restricted to a single continent).

2.4.1. Dispersal-reproduction trade-off

As each release experiment consisted of a random subset of individuals pooled from each iso-female line at the tunnel release point, the line identity was unknown for each recaptured fly. Thus, lines could not be distinguished (or nested statistically) within the dispersers and residents. We used R version 3.3.3 (R Core Team, 2012) and 'lme4' (Bates, Maechler & Bolker, 2012) to perform linear mixed effects analyses of the relationship between reproductive effort, reproductive fitness or time to first egg laying and dispersal category (resident versus disperse) for each species separately and also with all species pooled. As random effects, we explored the use of replicate (release trial number) with fixed effects of species, dispersal category or the interaction thereof to determine the best model, assessed using the lowest Akaike information criterion (AIC) scores. For all measures of reproductive output, the best model used replicate as a random effect and species as a fixed effect when species were pooled. To report which factors were significant in each analysis of each trait, I systematically tested for the effect of disperser, species and the interaction thereof, into different models with likelihood ratio tests of the full model with the effect in question against the model without the effect to obtain a p value. Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality.

To determine if a simple association existed in the magnitude of differences between the two dispersal groups among species, a Spearman rank correlation (in Statistica v13) was used to test the relationship between rank and difference in reproductive effort between resident and disperser flies within each species and in a separate correlation, the difference in reproductive fitness. To evaluate longevity between dispersal categories, a Cox-proportional hazards model was run in R (R core team, 2013, Vienna, Austria) using the 'survival' (Therneau and Grambsch, 2000) package and the data

used in SAS (version 9.4, Copyright © 2002-2012) to extract the lethal time where 50% of the individuals in a population had died (LT50).

2.4.2. Thermal flight performance

To test the effect of species, sex and temperature assayed on the flight responses in all 11 *Drosophilidae* species measured, a GLZ with binomial distribution and logit link function was run (Statistica v13). I also tested for correlations between body mass and wing area for each species to determine the relationship between morphological traits. A binomial GLZ was run in Statistica to determine the influence of individual size-related traits (body mass (Mb), wing area and wing loading [calculated as body mass/wing area]) on the flight responses of species at different temperatures. To determine the minimal adequate model, a modelling approach was undertaken in the 'MuMin' package in R using the 'dredge' function. In each case, the maximal, fully saturated model was run using a GLM with a binomial distribution and a logit link function, and thermal flight response as dependent variable, with morphology (Mb, wing area and wing loading), sex, species and test temperature and their interactions, to determine what subsets or best combinations of factors most strongly influenced flight response. Three separate sets of these models were run, once each for each of the different morphological predictors (Mb, wing area and wing loading respectively), and information criteria (AICc and BIC) used to rank models and their respective fits.

Moreover, TableCurve2D (<http://www.sigmaplot.co.uk/products/tablecurve2d/tablecurve2d.php>) was used to fit a non-linear curve (best-fit line) to my thermal flight performance data for each species separately. From the best-fit line (peak: all equations) I extracted the percentage maximum flight performance (Umax), the optimal temperature (Topt, in °C) and the performance breadth (Tbr) to obtain key thermal performance curve parameters for each species. To determine the relationship between rank, Tbr, Topt, Umax, mean body mass (Mb), wing area and wing loading, I ran a Spearman rank correlation in Statistica v13. I was also interested in the influence of the phylogenetic relationships between the different species on the traits examined, and how this may have been associated with any trait variation patterns observed in my data. To do this, DNA sequences (obtained using COI primer) were edited using the BioEdit Sequence Alignment editor 7.0.5 (Hall, 2005) and aligned in MAFFT (Kato et al., 2017). A Bayesian phylogeny was constructed in MRBAYES v3.2 (Ronquist et al., 2012) using sequence evolution models determined in JMODELTEST2 (Darriba et al., 2012) based on BIC scores. In 'caper' (R core team, 2013), the resultant phylogeny was compared to wing area, wing loading, body mass, Umax, thermal optimal temperature and thermal performance breadth using a phylogenetic generalized least square regression (pGLS) analysis fitted using maximum likelihood ('ppls' function; Freckleton et al., 2002) to determine whether any significant

effects remained when evolutionary relationships amongst species were accounted for. I used Pagel's λ (Pagel, 1999) as a quantitative measure for estimating phylogenetic signal. Pagel's λ scales between zero and one, with values closer to zero showing no phylogenetic signal and values closer to 1 showing strong phylogenetic signal for the traits. Furthermore, the 'contmap' function ('phytools' package; <http://www.phytools.org/>) was used to visualise and graphically illustrate traits on the phylogeny.

3. Results

3.1. Dispersal-reproduction trade-off

When species were analysed separately, the reproductive output results (Table 2) showed that in only one species, *Drosophila melanogaster*, dispersers had significantly fewer surviving adults than residents, i.e. lower reproductive fitness ($df=1$, $X^2=5.583$, $p=0.018$), while none of the other species or traits showed any statistically significant differences (Table 2). When species were pooled, for all traits scored, the best model included species as a fixed effect and replicate as a random effect. In these cases the dispersal category was found to be non-significant, although species (Table 3) differed significantly for reproductive effort ($df=4$, $X^2=185.53$, $p<0.001$), reproductive fitness ($df=4$, $X^2=335.87$, $p<0.001$) and time to first egg laying ($df=4$, $X^2=17.946$, $p<0.001$). Interactions between dispersal category were also not significant. Since only one trait in one species (of five species assessed) showed a significant trade-off in any of the traits examined (Figure 5c) I consider there to be weak overall support for the hypothesis of dispersal-reproduction trade-offs. In the case of *D. melanogaster*, the trade-off appeared to have a lasting impact as disperser flies also had significantly fewer adult offspring than the residents (Figure 5g). Two species, *D. simulans* (Figure 5d) and *D. immigrans* (Figure 5e) experienced increased egg numbers but this effect was not statistically significant. Interestingly, although *D. busckii* took the longest to lay eggs, it laid the most eggs and had the highest number of offspring (i.e. high reproductive effort and fitness). *Drosophila ananassae* performed the poorest by laying the least number of eggs and producing the lowest number of adults overall. *Drosophila busckii* (Figure 5m) took the longest time to lay eggs while *D. simulans* took the shortest time (Figure 5n).

Survival did not vary significantly between residents and dispersers ($df=1$, $X^2=0.428$, $p=0.513$; Figure 6). However, survival differed significantly between species ($df=4$, $X^2=106.735$, $p<0.0001$) in which *D. busckii* lived the longest and *D. immigrans* lived for the shortest time. Across species, the magnitude of the dispersal-reproduction trade-off, calculated as the difference in reproductive effort between disperser and resident flies, showed no correlation with geographic range size ranking ($r=-0.20$, $p=0.747$) and, similarly, for the magnitude of the trade-off in reproductive fitness between dispersers and residents with geographic rank ($r=-0.10$, $p=0.873$).

Table 2: Summary statistics of linear mixed effects analyses (including the random effect of replicate, in all cases) comparing dispersal group (disperser or resident) for reproductive effort, reproductive fitness and time to first egg laying in five *Drosophila* species. Significant effects are indicated by bold font.

Species	trait	df	X ²	p
	Reproductive effort	1	0.009	0.924
<i>D. ananassae</i>	Reproductive fitness	1	0.138	0.710
	Time to first egg laying	1	1.146	0.284
	Reproductive effort	1	1.969	0.160
<i>D. busckii</i>	Reproductive fitness	1	0.028	0.866
	Time to first egg laying	1	0.000	0.996
	Reproductive effort	1	0.149	0.699
<i>D. immigrans</i>	Reproductive fitness	1	0.635	0.426
	Time to first egg laying	1	0.041	0.839
	Reproductive effort	1	1.209	0.272
<i>D. melanogaster</i>	Reproductive fitness	1	5.583	0.018
	Time to first egg laying	1	0.087	0.769
	Reproductive effort	1	0.849	0.357
<i>D. simulans</i>	Reproductive fitness	1	1.300	0.254
	Time to first egg laying	1	0.051	0.822

Table 3: Results of the best-fit, mixed effects model for each reproductive output trait with species as a fixed effect. Replicate was included as a random effect for reproductive effort and reproductive fitness, and time to first egg laying. All results are significant at 5% confidence interval.

Trait	df	χ^2	p value
Reproductive effort	3	185.01	<0.001
Reproductive fitness	3	335.55	<0.001
Time to first egg laying	3	517.55	<0.001

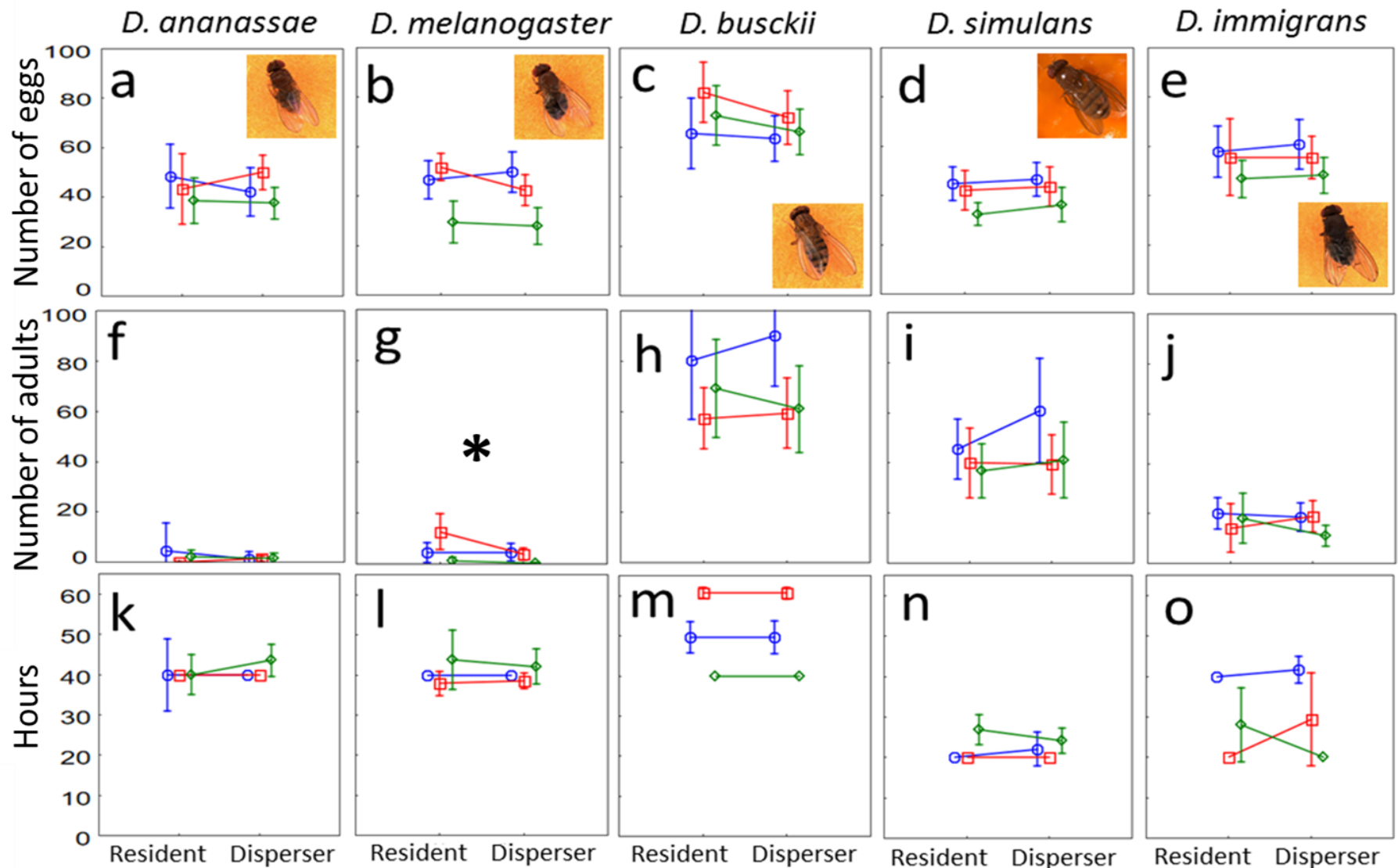


Figure 5: Summary results of the weighted mean (\pm standard error) of each experimental replicate performed for reproductive effort (a-e), reproductive fitness(f-j) and time taken to lay the first clutch of eggs (k-o) for each *Drosophila* species categorized into disperser and resident flies (see Methods section for details). The rows represent the traits measured and the columns represent the species related to the trait. Replicates are in different colours (blue, red, green). The asterisk indicates significance.

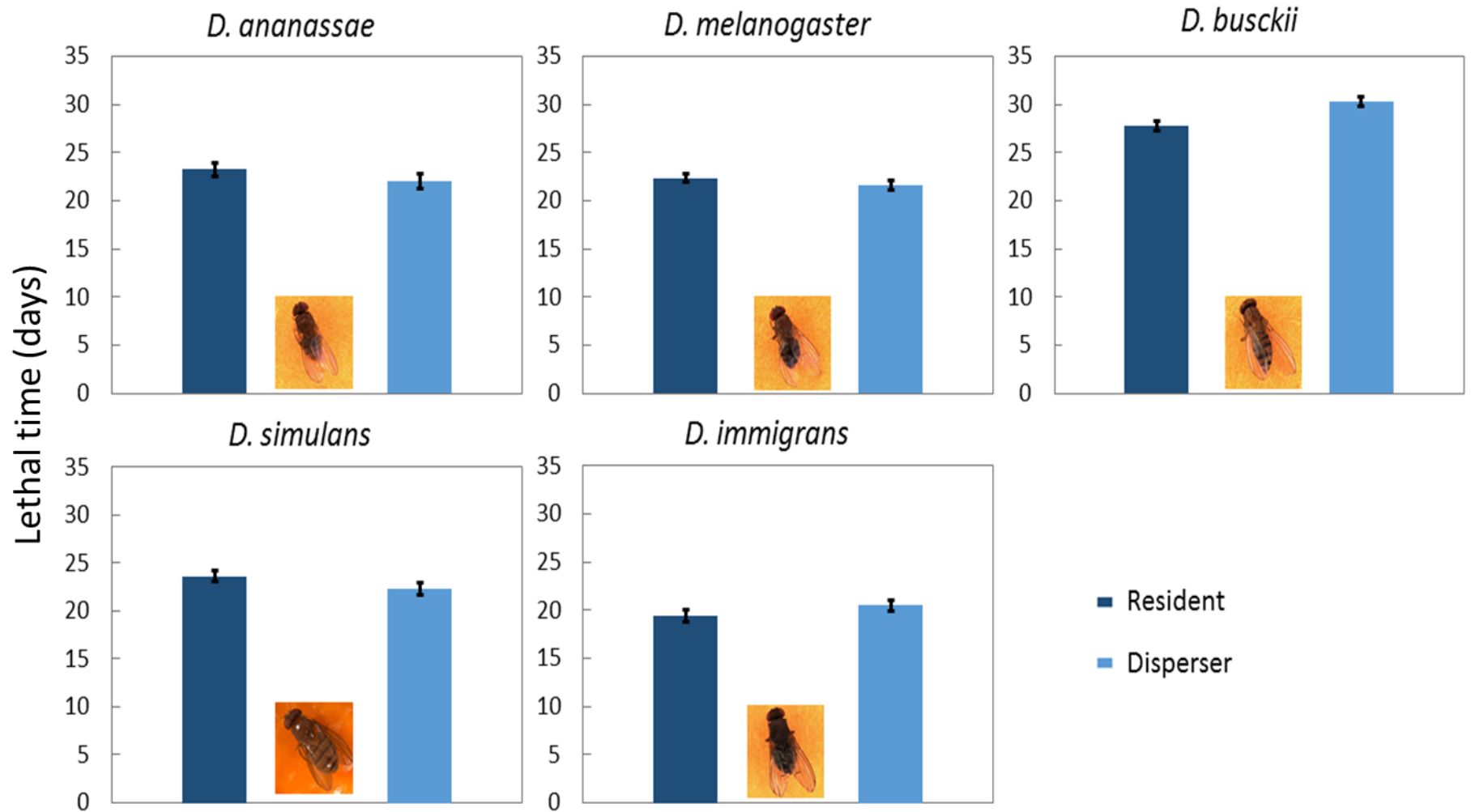


Figure 6: Lethal time in days for 50% (LT50) of the females per species for dispersers and residents of each of the five *Drosophila* species investigated.

3.2. Thermal flight performance

Thermal flight performance was not influenced by sex or species but was influenced by experimental temperature across all species (Table 4). However, this general temperature effect on flight was largely driven by the significant effects of temperature which were found only in *D. hydei* (df= 3, $X^2= 17.446$, $p<0.001$), and *D. funebris* (df=3, $X^2= 24.706$, $p<0.001$) but not in the other species when examined in isolation. Although the thermal flight response did not differ between sexes across all species, *Z. taronus* (df=1, $X^2= 61.944$, $p<0.001$) showed significant sex effects at certain temperatures (20°C and 28°C). *Drosophila immigrans* showed some variation between sexes in response to these temperatures however they are not statistically significant (df=1, $X^2= 1.978$, $p=0.160$) and there was a mixed effect across the experimental temperatures tested (Figure 7). The females of *Z. taronus* performed significantly better at 20°C and 28°C compared to their male counterparts, while *D. immigrans* females performed worse than *D. immigrans* males at 20°C and 28°C.

Body mass, wing area and the interaction between them were significantly correlated among individuals from all species ($r = 0.7699$, $p<0.001$) (Figure 8). Within each species examined separately, I always found a significant positive correlation between body mass and wing area (*D. hydei*: $r=0.7444$; *D. funebris*: $r=0.7552$; *D. busckii*: $r=0.7335$; *Z. taronus*: $r=0.7037$; *Z. vittiger*: $r=0.5442$; *D. immigrans*: $r=0.7127$; *D. simulans*: $r=0.7535$; *Z. indianus*: $r=0.4611$; *Z. tuberculatus*: $r=0.5407$; *D. melanogaster*: $r=0.6021$; *D. ananassae*: $r=0.6782$) indicating that as body mass increases so does wing area. Strong positive correlations were found between U_{max} and T_{br} ($r=0.736$, $p=0.010$), body mass and wing area ($r=0.927$, $p=0.006$); and body mass and wing loading ($r=0.764$, $p< 0.001$) (Table 5). These results indicate dependence of one trait on the other since the one increases as the other increases, although any direction of causality, if at all, remains unclear.

Flight responses were not influenced by the morphology of flies as measured by body mass, wing area and wing loading in all species (Table 6). There were no obvious or marked systematic morphological differences between flies tested and categorised into those that successfully flew ('fliers') and those that failed to fly ('failure'), when compared at each test temperature, either within or across species (Figures 9-11). Intraspecifically, *D. immigrans* and *Z. taronus* have large variability among individuals in body mass while *D. busckii* and *Z. indianus* have small variability in body mass (Figure 9). While there are no obvious species trends for having much variability in wing area and wing loading, *D. busckii* shows the lowest variability in wing area (Figure 10) and *Z. indianus* has the least variability in wing loading among individuals (Figure 11). However, when running all possible iterations

of factors influencing thermal flight performance, including sex, species, temperature, wing area, body mass, wing loading and their interactions, the best fit model was one that included temperature and wing area but not any other predictors or interactions among them (Table 7). In other words, flies flew more readily at warmer temperatures and with greater wing area.

To investigate the possible influence of phylogeny on performance measures I explored 32 different pGLS models (Table 8) and, based on AICc scores and Akaike weights, single predictor models were always considered the best models of those tested. However, the first (best) 9 models could not be differentiated on the basis of model probability as AICc varied little among these. This was also the case when we followed a minimal adequate model approach. For the three best models (T_{opt}, T_{br}, U_{max}) phylogenetic signal was found to be non-significant ($\lambda=0.00$) for any of the traits examined (Table 9). The traits (T_{opt}, T_{br}, U_{max}, body mass, wing area and wing loading) were overlaid with phylogeny for a graphical representation of the interspecific differences or lack thereof (Figure 12a-f). *Drosophila busckii* and *D. funebris* have the lowest T_{opt} (Figure 12a), indicated by the yellow bars, which means they perform best at lower temperatures, yet they have the greatest T_{br} (Figure 12f). U_{max} differences are not clade specific; *D. funebris* has the highest U_{max} (Figure 12b). Body mass (Figure 12d) and wing loading (Figure 12e) are highest for *Z. taronus*, *Z. vittiger* and *D. immigrans* while wing area is highest for the *Zaprionus* clade (Figure 12c).

Table 4: Summary results of the exploratory GLM model for species flight response with species, sex and temperature effects. Significance is indicated with bold font.

	df	Wald X²	p
Intercept	1	12.324	<0.001
Species	10	7.985	0.630
Sex	1	0.009	0.925
Temperature	1	10.723	<0.001
Species x Sex	10	5.120	0.883
Species x Temperature	10	8.543	0.576
Sex x Temperature	1	0.058	0.810
Species x Sex x Temperature	10	6.557	0.767

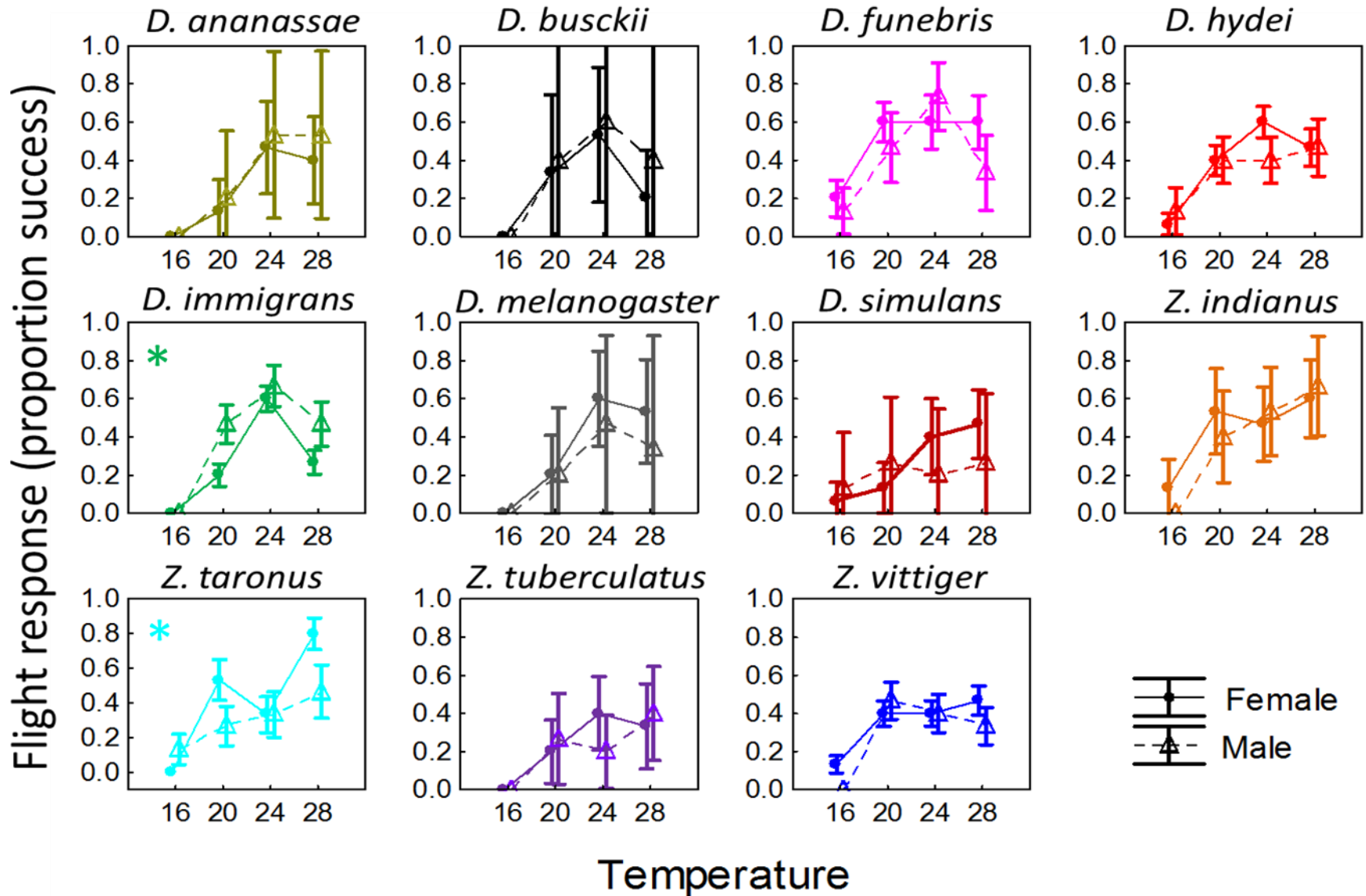


Figure 7: Summary results of the model's weighted mean \pm standard error of thermal flight response curves in males and females of each Drosophilidae species. The asterisk indicates a significant difference in response between the sexes.

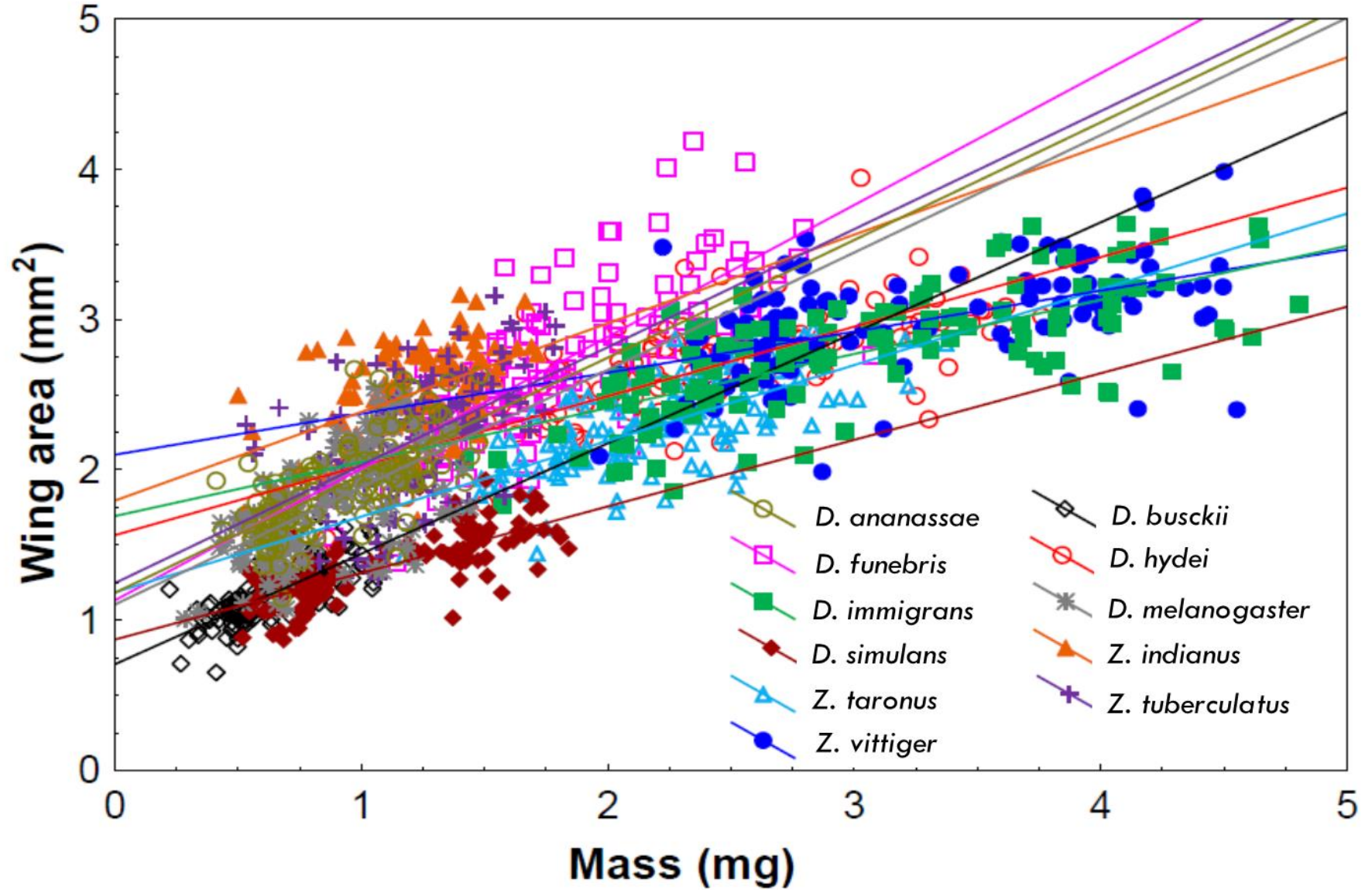


Figure 8: Scatterplot showing the relationship between body mass and wing area for 11 Drosophilidae species from the thermal flight response experiments.

Table 5: Results of a Spearman rank correlation test of species' geographic distribution ranking (where higher rank equals broader geographic distribution), thermal performance breadth (Tbr), optimal performance temperature (Topt, in °C), the flight response scored as flight performance at Topt (Umax), mean body mass in mg (Mb), wing area (mm²) and wing loading (mg/mm²). Significance at 5% confidence interval ($p > 0.05$) is indicated by bold font.

Variable	Rank	Tbr	Topt (°C)	Umax	Mb (mg)	Wing area (mm ²)	Wing loading (mg/mm ²)
Rank	1.000	0.073	-0.092	0.146	-0.255	-0.264	0.027
Tbr		1.000	-0.422	0.736	0.418	0.582	0.182
Topt (°C)			1.000	-0.193	-0.128	-0.275	-0.294
Umax				1.000	0.136	0.300	0.073
Mb (mg)					1.000	0.927	0.764
Wing area (mm ²)						1.000	0.564
Wing loading (mg/mm ²)							1.000

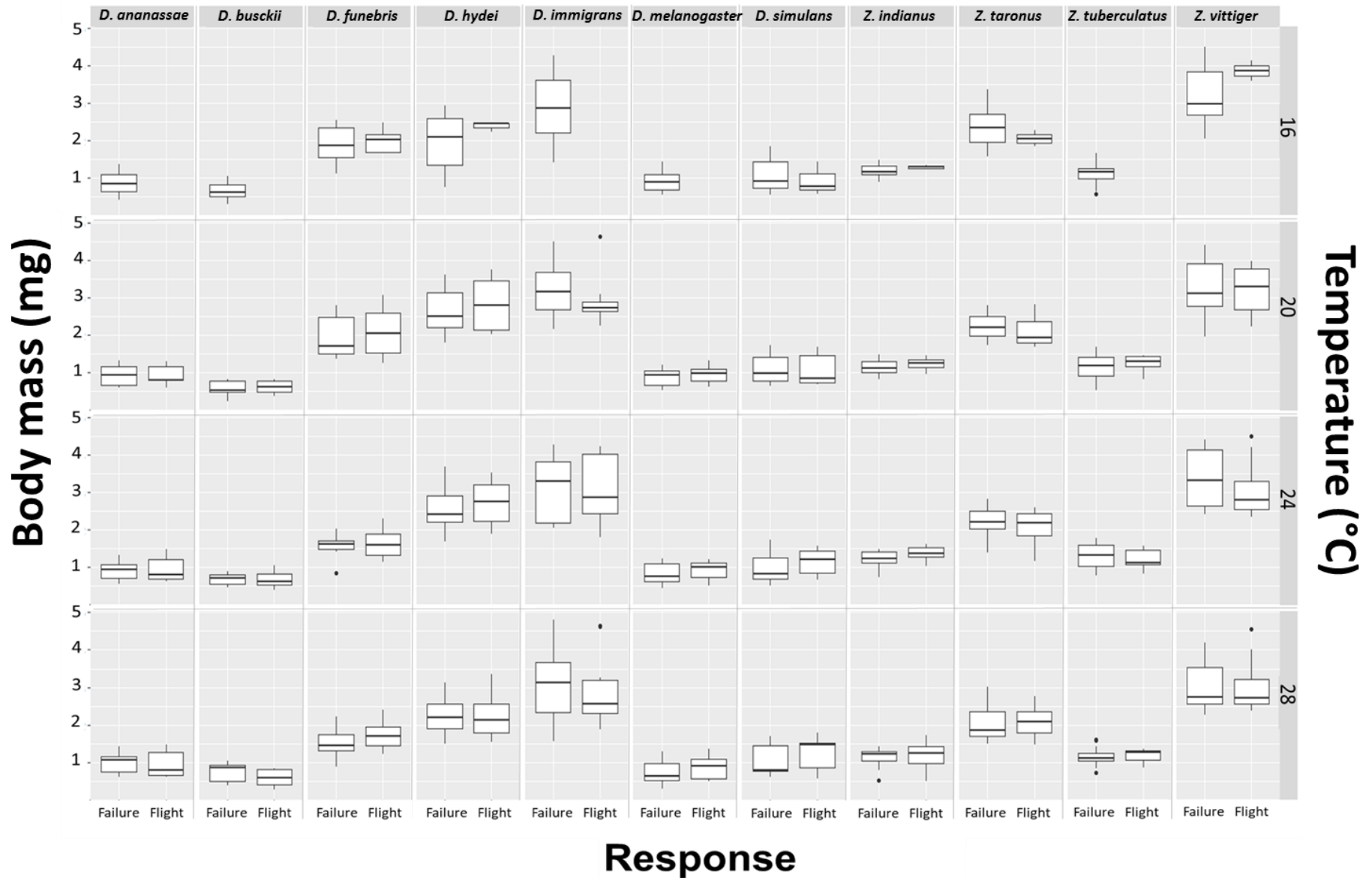


Figure 9: Body mass (mg) of the 11 different Drosophilidae species that flew or failed to fly at the different experimental temperatures on the thermal stage. There are no significant differences.

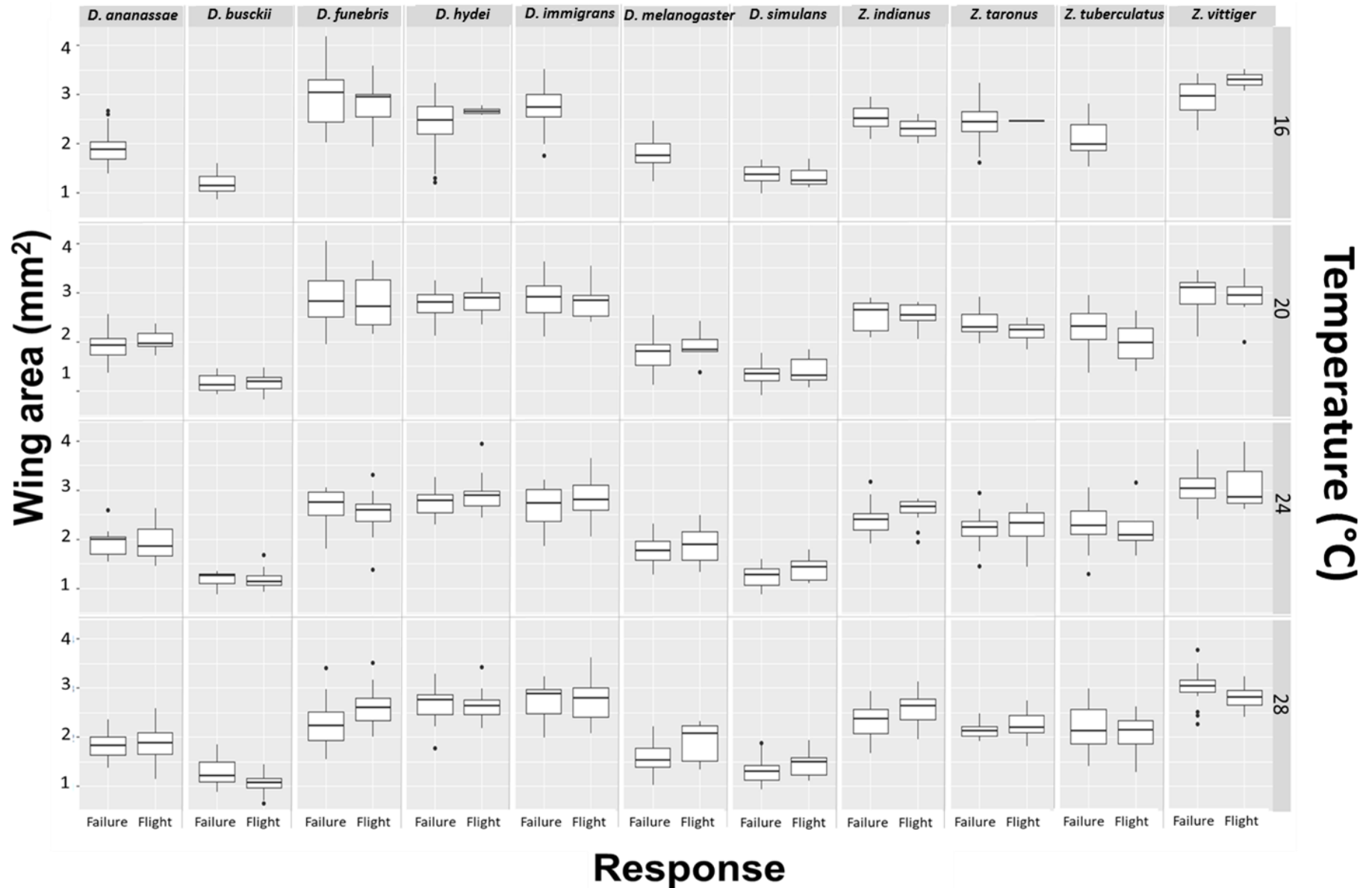


Figure 10: Wing area (mm²) of the 11 different Drosophilidae species that flew or failed to fly at the different experimental temperatures on the thermal stage. There are no significant differences.

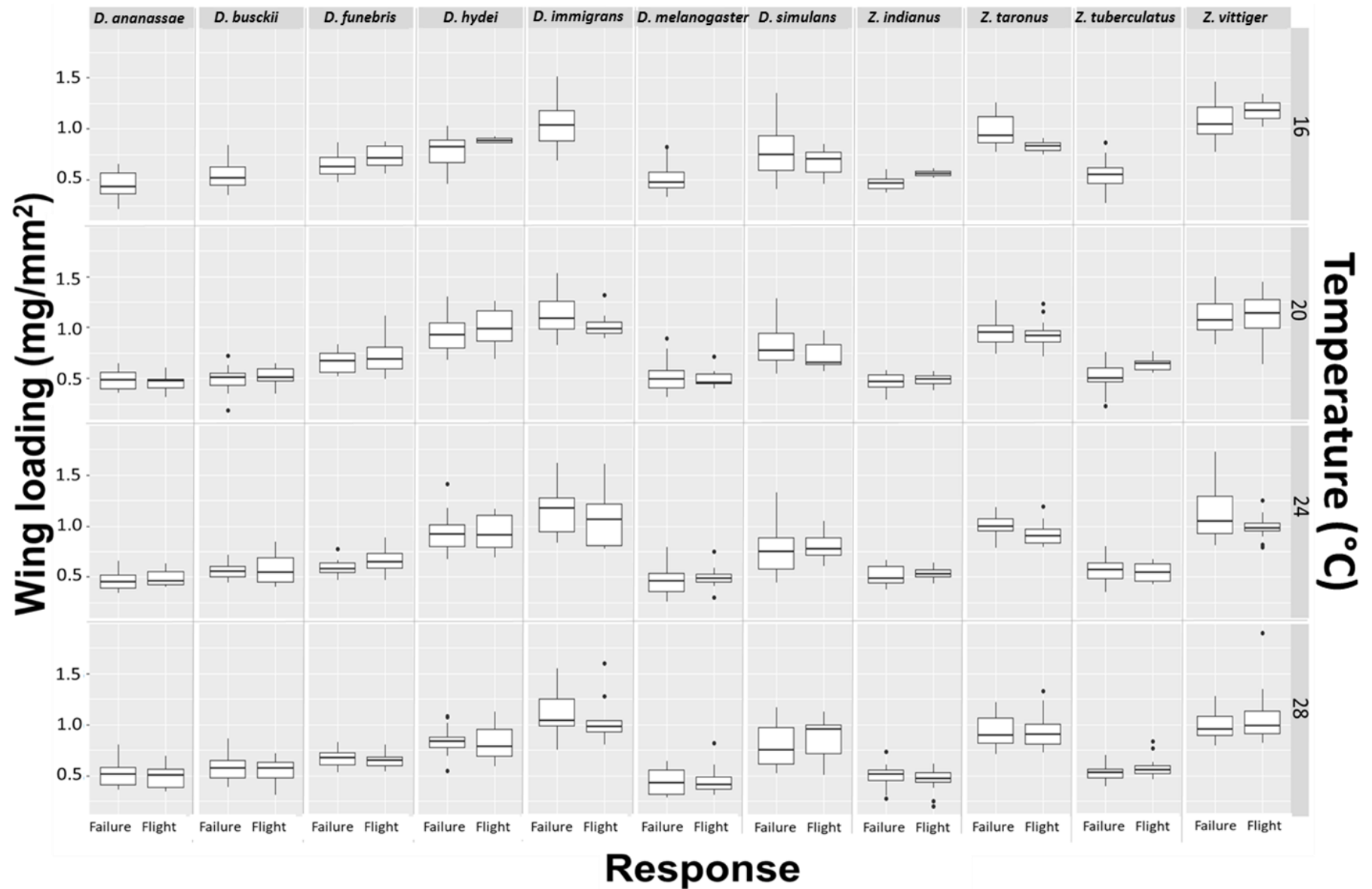


Figure 11: Wing loading (mg/mm²) of the 11 different Drosophilidae species that flew or failed to fly at the different experimental temperatures on the thermal stage. There are no significant differences.

Table 6: Results of the GLM model for all Drosophilidae species flight response with mass, wing area, wing loading and temperature as a continuous predictor. No significant effects were detected at 5% confidence interval ($p > 0.05$).

Species	Mass (mg)			Wing area (mm ²)			Wing loading (mg/mm ²)		
	Estimate ± Std. Error	z value	p	Estimate ± Std. Error	z value	p	Estimate ± Std. Error	z value	p
Intercept	-9.14 ± 5.3	-1.731	0.083	-6.80 ± 8.9	-0.767	0.443	-10.62 ± 6.4	-1.649	0.099
<i>D. ananassae</i>	0.69 ± 7.8	0.088	0.930	2.00 ± 5.9	0.341	0.733	1.47 ± 17.6	0.083	0.934
<i>D. busckii</i>	2.80 ± 7.7	0.365	0.715	5.08 ± 7.4	0.691	0.490	-2.02 ± 15.7	-0.128	0.898
<i>D. funebris</i>	-5.17 ± 5.9	-0.871	0.384	-4.37 ± 5.0	-0.873	0.383	7.14 ± 16.7	0.427	0.669
<i>D. hydei</i>	0.25 ± 5.8	0.044	0.965	4.34 ± 5.7	0.760	0.447	3.26 ± 14.8	0.220	0.826
<i>D. immigrans</i>	-2.51 ± 5.6	-0.450	0.653	-0.59 ± 5.4	-0.109	0.913	-6.90 ± 14.1	-0.488	0.626
<i>D. melanogaster</i>	-5.41 ± 7.5	-0.718	0.473	-2.37 ± 6.2	-0.380	0.704	-13.32 ± 16.6	-0.804	0.421
<i>D. simulans</i>	-6.36 ± 6.2	-1.018	0.309	-1.18 ± 7.4	-0.159	0.873	-16.01 ± 14.3	-1.122	0.262
<i>Z. indianus</i>	3.57 ± 7.9	0.450	0.653	-6.60 ± 6.3	-1.046	0.296	18.95 ± 19.6	0.965	0.335
<i>Z. taronus</i>	-6.68 ± 6.2	-1.078	0.281	-5.15 ± 6.0	-0.855	0.393	-16.20 ± 15.6	-1.040	0.298
<i>Z. tuberculatus</i>	-0.69 ± 7.8	-0.088	0.930	-2.00 ± 5.9	-0.341	0.733	-1.47 ± 17.6	-0.083	0.934
<i>Z. vittiger</i>	-2.154 ± 5.6	-0.383	0.702	5.11 ± 5.8	0.888	0.375	-10.49 ± 14.1	-0.744	0.457

Table 7: Summary of the coefficients and results for the overall best-fit model for flight response obtained from the 'dredge' function in the 'MuMin' package in R. All effects presented below are significant.

	Estimate	Std. Error	z value	p
Intercept	-4.0644	0.3355	-12.113	<0.001
Temperature	0.1070	0.0116	9.226	<0.001
Wing area	0.2151	0.0731	2.941	<0.01

Table 8: All models considered in the phylogenetic generalized least square regression (pGLS) analyses to predict species geographic distribution rank (dependent variable), ranked according to AICc (small sample size Akaike Information Criterion). logLik is the log likelihood. Delta is the change in model AICc relative to the best (lowest AICc) model.

Model	logLik	AICc	delta	Akaike weight
Tbr	-24.247	54.209	0.000	0.1334
Umax	-24.297	54.308	0.100	0.1269
Topt	-24.366	54.446	0.238	0.1184
log(Mb)	-24.469	54.652	0.443	0.1069
log(Wing loading)	-24.507	54.729	0.521	0.1028
log(Wing area)	-24.527	54.768	0.560	0.1008
Wing loading	-24.538	54.790	0.581	0.0997
Mb	-24.553	54.820	0.612	0.0982
Wing area	-24.560	54.835	0.626	0.0975
Umax x Wing loading	-23.316	62.631	8.423	0.0020
Umax x Wing loading	-23.316	62.631	8.423	0.0020
Tbr x Wing area	-23.499	62.999	8.790	0.0016
Topt x Tbr	-23.770	63.540	9.332	0.0013
Topt x Mb	-23.847	63.694	9.485	0.0012
Tbr x Mb	-23.954	63.908	9.699	0.0010
Topt x Wing area	-24.031	64.062	9.854	0.0010
Wing loading x Mb	-24.051	64.101	9.893	0.0009
Wing area x Mb	-24.063	64.125	9.917	0.0009
Topt x Umax	-24.117	64.233	10.025	0.0009
Tbr x Umax	-24.124	64.247	10.038	0.0009
Umax x Mb	-24.171	64.343	10.134	0.0008
Topt x Wing loading	-24.213	64.425	10.216	0.0008
Topt x Tbr x Wing loading	-8.464	176.928	122.719	<0.0001
Topt x Umax x Wing area	-12.046	184.091	129.883	<0.0001
Tbr x Umax x Wing loading	-12.128	184.255	130.047	<0.0001
Topt x Umax x Wing loading	-14.702	189.403	135.195	<0.0001
Topt x Tbr x Wing area	-17.372	194.745	140.536	<0.0001
Topt x Tbr x Mb	-17.456	194.912	140.703	<0.0001
Tbr x Umax x Wing area	-17.880	195.760	141.551	<0.0001
Tbr x Umax x Mb	-18.862	197.724	143.516	<0.0001
Topt x Tbr x Umax	-21.378	202.756	148.548	<0.0001
Topt x Umax x Mb	-21.695	203.391	149.182	<0.0001

Table 9: Summary of the three best single predictor models used to predict geographic distribution rank from thermal flight performance curve parameters, including Pagel's λ to show the lack of phylogenetic signal for the traits. No significant effects were detected at 5% confidence interval ($p > 0.05$).

Model	df	estimate	Std. error	t value	p	λ
Tbr	2	0.742	0.987	0.752	0.474	0.00
Umax	2	5.305	7.653	0.693	0.508	0.00
Wing loading	2	-1.070	3.742	-0.286	0.782	0.00

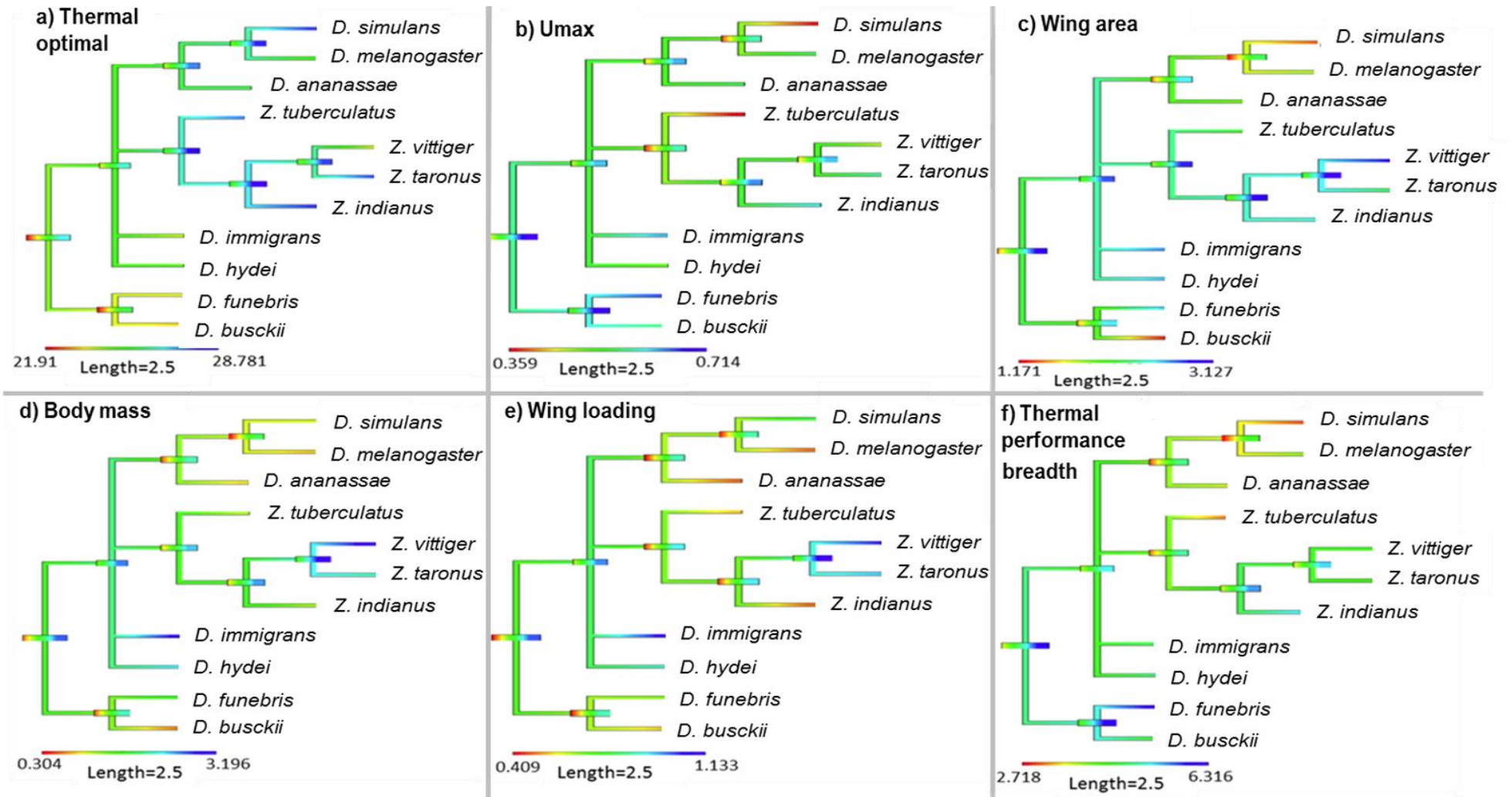


Figure 12: Phylogenetic tree of 11 Drosophilidae species included in my study overlaid with a) their optimal thermal temperature of flight performance, b) Umax of flight, c) wing area, d) body mass, e) wing loading and f) thermal performance breadth of flight. The bars at the nodes represent the standard error at the nodes while colours towards the red end of the scale indicate lower trait values compared to the blue end of the scale.

4. Discussion

4.1. Dispersal-reproduction trade-offs

Here I investigated whether a systematic dispersal-reproduction trade-off is present in Drosophilidae species and, if so, whether it relates to their respective geographic distributions. More specifically, I was interested in 1) whether there was a dispersal-reproduction trade-off between narrow- and widely-distributed species and how common it might be, and 2) whether the magnitude of the dispersal-reproduction trade-off differed between narrow and widely-distributed species. Reproduction-related trade-offs in drosophilids have mainly been studied in the context of dietary macronutrients (e.g. carbohydrates vs. lipids) (Mason et al., 2018), thermal variability (Marshall and Sinclair, 2010; Ryan et al., 2016) or hormone regulation (Flatt and Kawecki, 2007) but none, to my knowledge, have considered dispersal-reproduction trade-offs as the major focal study objective. My results showed two major findings given my study objectives. First, there was only weak support for the dispersal-reproduction trade-off as only one of the five species (*Drosophila melanogaster*) tested showed statistically significant evidence of the trade-off in the direction expected for the trait of reproductive fitness, while the remaining four species showed no significant differences in reproductive performance between disperser and resident flies. There was also great variation in outcomes among the reproductive traits scored, showing that the method used to measure such trade-offs will potentially influence the study outcomes (see discussions in Hoffmann and Ross, 2018; Hoffmann and Sgro, 2018). Furthermore, the dispersal-reproduction trade-off is unlikely to aid insect invasion success or be more generally associated with niche breadth or geographic distribution as *D. melanogaster* showed evidence of the trade-off and *D. simulans*, both cosmopolitan species, did not.

There was no evidence for a clear dispersal-reproduction trade-off when considering geographic distribution in all the Drosophilidae species included in this study. However, for *D. melanogaster*, more adults emerged from the eggs laid by the residents suggesting, at least in this species, that the trade-off might translate into an evolutionary fitness consequence. Counter to our expectation that broadly-distributed species would lack the dispersal-reproduction trade-off, or perhaps that more specialized, narrow-range species would show a more pronounced trade-off, *D. melanogaster* provided some weak support for the trade-off where reproductive fitness was lower after dispersal. However, given the broad lack of a systematic effect in my results, it can then be concluded that dispersal and any associated effects on reproductive output do not enhance the ability for a particular *Drosophila* species to be widely-distributed and, vice versa, that the trade-off likely does not limit species with more narrow or specialized distributions.

The dispersal-reproduction trade-off has been shown in wing-dimorphic species, wing-monomorphic species (Malmqvist, 2000; Elkin and Reid, 2005; Khuhro et al., 2014), and in some instances, plants (Tabassum and Leishman, 2018). Research investigating the dispersal-reproduction trade-off in wing-dimorphic insects has shown a significant decrease in reproduction for dispersing crickets (Tanaka and Suzuki, 1998; Langellotto et al., 2000; Zeng and Zhu, 2012) and pygmy grasshoppers (Steenman et al., 2015) relative to non-disperser morphs. The same pattern has also been shown in the African armyworm moth (*Spodoptera exempta*) (Gunn et al., 1989) and the beet armyworm (*S. exigua*) (Jiang et al., 2010), which, like the Drosophilidae in my study, are not wing-dimorphic. Very little information however, is available on the dispersal-reproduction trade-off in wing-monomorphic species, specifically how this relates to geographic distribution, and with contrasting results between species in my study, it seems that the presence of the trade-off is species specific and unrelated to their geographic distribution. An alternative explanation for the lack of the trade-off may be due to food availability post-dispersal. This was shown in mountain pine beetles (Elkin and Reid, 2005), where the abundance of food after dispersal lead to no decrease in reproductive ability after the beetles dispersed up to 50m. This could be what allowed for the disperser drosophilids to eliminate the trade-off, as food availability was not controlled in my study and instead was available ad libitum. It would perhaps be of further interest to restrict food resources and reassess the trade-off between different Drosophilidae species, or assess multiple interactive stressors more generally, on the outcome of tests of the dispersal-reproduction trade-off hypothesis.

Furthermore, I show no significant fitness costs, in terms of longevity between dispersers and residents or across species geographic distributions. This shows that dispersal appears to have little to no effect on overall life-history traits for *Drosophila* of any given geographic range size, which perhaps simply suggests that *Drosophila* are well adapted to disperse and reproduce. Dispersal-related life-history costs have been documented in other insect taxa such as damselflies (Chaput-Bardy et al., 2010) and lacewings (Khuhro et al., 2014) but no work to date has been published relating to *Drosophila*.

4.2. Thermal flight response

I determined the thermal flight response of 11 species in the Drosophilidae family, and measured potentially correlated morphological traits commonly associated with flight ability, to determine an individual's response. I was primarily interested in 1) variation in flight responses between narrow and widely-distributed Drosophilidae at different temperatures; 2) morphological traits (body mass, wing area, wing loading) accounting for flight and 3) accounting for phylogeny in these trait differences. Aspects of TFPCs differed between the 11 drosophilid species examined here, as might be generally expected. However, the ranking of geographic range extent (breadth of distribution) was poorly

predicted by a range of TFPC parameters, and phylogenetic association failed to contribute meaningful variation to explain such flight responses across the thermal range employed here.

The thermal performance curves generated for each species provide novel data that are not readily available in the literature. Thermal locomotor responses have been determined in selected *Drosophila* species (Gilchrist et al., 1997; Gibert et al., 2001; Dillon and Frazier, 2006; Latimer et al., 2011) but the thermal flight response of most of the *Drosophila* species used in my study has not previously been determined. The trend found among my species was that flight response decreases as temperature decreases. Of the temperatures used, most species reached their thermal optimal performance at 24°C, which resulted in fewer responses at the higher 28°C; this trend is most visible in both sexes of *D. busckii*, *D. immigrans*, *D. melanogaster*, females of *D. hydei*, *D. ananassae* and *Z. tuberculatus*, and *D. funebris* males. The optimal temperature for certain species (*Z. taronus*, *D. simulans* females, *D. hydei* males, *Z. tuberculatus* males and *Z. indianus* males) have not been reached with the temperatures that were used as the 28°C had the highest performance or proportion of the population flying. Dillon and Frazier (2006) found that flight in *D. melanogaster* decreased with decreased temperature, which is the same trend in all the species investigated here. Mass and wing area are positively correlated which was again largely expected, however, that the best model was one that used temperature and wing area as predictors, is perhaps a more novel result.

Morphological traits have shown to influence locomotor ability (De Bie et al., 2012, Reim et al., 2018) and thus body mass, wing area and wing loading were considered in my study. These traits were shown to not have an effect on thermal flight response and this result is supported by Dillon and Frazier (2006) who found mass to have no significant influence on flight ability while Reim et al. (2018) found wing loading, among other traits, to have a significant influence on flight ability. To explain the geographic distributions of the different species, different parameters (Topt, Tbr, Umax) of the thermal performance curve were considered but only at the species level, and given my study's experimental design, could not be determined at the individual level within each species. The Umax, Tbr, Topt was not significant between species. Species of lowest (specialised) ranking were found to have the highest Topt, which corresponds with their known climatic geodistributions of Afrotropical (*Z. taronus*, *Z. vittiger* and *Z. tuberculatus*) (Bächli, 2018). Overall, the TPC parameters cannot be used to explain the geographic distribution of drosophilid species, which is perhaps unexpected (see e.g. Overgaard et al., 2014; Bush et al., 2016).

In a review by Dillon et al. (2009) it was emphasised that developmental temperature can influence the thermal preference of some species (*D. melanogaster*) yet have no effect on other species (e.g. *D. simulans*). The preferred temperature of Drosophilidae can also be different at different stages of the adult life stage (Yamamoto and Ohba, 1982; Yamamoto and Ohba, 1984; Sayeed

and Benzer, 1996). Thus, my results may be specific to 6-day-old adults only and could be different if examined across a broader age range. Phylogeny was then tested to determine whether it explains the TPC parameters. My results reflect that phylogeny does not appear to account much for any of the traits measured. This indicates that the statistical models did not need to account for phylogeny in this case. However, the species I have are all closely related when looking at the greater Drosophilidae phylogeny (Kellerman et al., 2012) which may have biased this result with its small-scale phylogenetic differences and relatively low sample sizes. This seems to be the first work that integrates these traits with the Drosophilidae phylogeny, so these results cannot be readily compared with other studies.

4.3. Implications for distributions and invasions

“There are some core issues around physiological limits for which we currently have limited data in Drosophila and other insects; these include the impact of multiple stressors and biotic interactions on physiological limits, the impact of extremes on limits and species distributions and the effect of gene flow on geographical limits.” Hoffmann, 2010, p. 828.

As mentioned in the quote above from Hoffmann (2010), there is little known about the impacts of various physiological factors that contribute to current range distributions or future range expansions or invasions. This study provides a contribution towards these aspects by providing information on species thermal performance and addressing the knowledge gap about dispersal costs for Drosophilidae. My findings indicate that by dispersing across 20m, Drosophilidae experience no major or obvious reproduction costs and can ultimately spread at least 20m from a point of introduction while having the ability to successfully establish a population under suitable environmental conditions. According to the TFPCs, all species fly well in warmer conditions and this suggests that these species will be able to expand or shift their geographic ranges with increased temperatures should other necessary survival resources be accessible. This is only considering temperature as a variable while other factors which weren't considered, such as desiccation and behaviour, might also play a role (Kellermann et al., 2018).

4.4. Methodological limits of present study

The outcomes of this study were perhaps limited by prevailing weather conditions as a large portion of the flight tunnel (dispersal-reproduction trade-off) experiments were weather-dependent. Although I attempted to control this by only performing these experiments when the ambient temperature was greater than a 18°C or cooler than 30°C, the wind needed to be blowing at 3m.s⁻¹ or slower and the sky needed to be mostly clear or with minimal clouds otherwise the conditions were too cool/hot or too windy for the flies to disperse efficiently. Such conditions were rarely stable across the entire duration that the flies were in the tunnel. Environmental factors were

difficult to measure and incorporate into analyses for the dispersal-reproduction trade-off experiment, as these factors were highly variable: in other words, one species may have had to work hard to disperse while another may have had to make little effort to disperse in the tunnel, and this may, in part, be determining the results for each species.

The trap-and-capture method of multiple fruit varieties as well as mushrooms worked well for this project and attracted up to 15 different drosophilid species across the relatively low number of trapping sites used for this study. More specialised species could have been studied had the knowledge to rear more diet-specific species been readily available as well as live-capture methods for these species. A final suggestion by Kingsolver et al., (2011) regarding life-history trade-offs is to measure across different developmental phases and across generations before understanding the true trends for evolutionary fitness. Although there is limited data on *Drosophilidae* daily dispersal distance, McInnis et al. (1982) provide evidence that dispersal distance varies with species; distances can range between tens of metres to hundreds of metres. This finding could imply that the standard dispersal distance tested in this study may not have been far enough to display the dispersal-reproduction trade-off, especially for more willing disperser species. In future, perhaps a release recapture assay approach (e.g. Kristensen et al., 2008) can be employed to test the magnitude of the trade-offs across a broader range of conditions. A broader range of species and temperatures employed to test flight performance would also be useful in future.

4.5. Conclusions and future directions

To conclude, my results provide some, albeit rather weak, support for the presence of a dispersal-reproduction trade-off in five *Drosophila* species. There is little variation in thermal flight performance at different temperatures across the eleven species tested within the *Drosophilidae* family. These results indicate that reproduction-related costs due to dispersal is perhaps not a limiting factor for narrowly-distributed species, and on the other hand, the lack of trade-off probably does not aid widely distributed species to have a more general distribution pattern. Morphological traits such as body size do not correlate with geographic range size nor does the optimal temperature for flight performance. This indicates more complex ecological and climatic factors, such as microclimate opportunities (e.g. Pincebourde and Woods, 2012; Woods et al., 2015; Pincebourde and Suppo, 2016) need to be considered for small-sized insects. Therefore, this study suggests that the trade-offs are not as common as might have been expected when assessed systematically, at least not in the genus *Drosophila* and under the conditions employed here. Clearly, the extent of reproduction-dispersal trade-offs, and their magnitude and direction, require further investigation across a broader suite of species and conditions. Future studies should consider testing flight assays under various stressful conditions (e.g. nutrient restriction) to attain

better insights into the potential fitness consequences. It would also be useful to explore these questions across a wider range of Drosophilidae species. Lester et al. (2007) suggest the importance of considering other processes that can contribute towards a species' range size other than simply dispersal ability. Consequently, this study could be extended by investigating the effect of additional environmental variables (e.g. niche or nutritional requirements, environmental variability and tolerance, age) on dispersal-reproduction trade-offs to help understand geographic range size variation (Lester et al., 2007) and potential range expansions under forecast climate change.

5. References

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