Flight-reproduction trade-offs are weak in a field cage experiment across multiple *Drosophila* species

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ABSTRACT

Flight-reproduction trade-offs, such that more mobile individuals sacrifice reproductive output (e.g., fecundity) or incur fitness costs, are well-studied in a handful of wing-dimorphic model systems. However, these trade-offs have not been systematically assessed across reproduction-related traits and taxa in wing monomorphic species despite having broad implications for the ecology and evolution of pterygote insect species.

Here we therefore determined the prevalence, magnitude and direction of flight-reproduction trade-offs on several fitness-related traits in a semi-field setting by comparing disperser and resident flies from repeated releases of five wild-caught, laboratory-reared *Drosophila* species, and explicitly controlling for a suite of potential confounding effects (maternal effects, recent thermal history) and potential morphological covariates (wing-loading, body mass).

We found almost no systematic differences in reproductive output (egg production), reproductive fitness (offspring survival), or longevity between flying (disperser) and resident flies in our replicated releases, even if adjusting for potential morphological variation. After correction for false discovery rates, none of the five species showed evidence of a significant fitness trade-off associated with increased flight (sustained, simulated voluntary field dispersal).

Our results therefore suggest that flight-reproduction trade-offs are not as common as might have been expected when assessed systematically across species and under the relatively standardized conditions and field setting employed here, at least not in the genus *Drosophila*. The magnitude and direction of potential dispersal- or flight-induced trade-offs, and the conditions that promote them, clearly require closer scrutiny.

We argue that flight or dispersal is either genuinely cheaper than expected, or the costs manifest differently than those assessed here. Lost opportunities (i.e., time spent on mate-finding, mating or foraging) or nutrient-poor conditions could promote fitness costs to dispersal in our study system and that could be explored in future.

Introduction

Dispersal is crucial for the ecology and evolution of organisms (Roff, 1992; Stearns, 1992). Movement, be it for daily home range maintenance, or for dispersal to colonize new habitats or recolonise patches previously occupied, is critical for population-level gene flow, to avoid intraspecific competition, locate food and mates and to escape threats, such as habitat disturbances, stressful environmental conditions or predators (Culik, 2001; Langellotto and Denno, 2001; Trakhtenbrot et al., 2005; Anderson et al., 2008; Bonte and Van Dyck, 2009). However, dispersal can involve considerable time and energy investment (Bonte et al., 2012) and may therefore affect species' performance and survival through trade-offs among key life-history, behavioural or physiological traits (Baker and Rao, 2004). Such dispersal costs can include reduced survival, lost opportunities and time spent in a

new area due to unfamiliarity (Part, 1991; Brown et al., 2008), lifestyle-specific traits (Bal et al., 2014), energy trade-offs (Nespolo et al., 2008), or direct movement costs of exploring the new environment (Vahl and Clausen, 1980; Basson et al., 2017), and, importantly, reduced fecundity or lifetime reproductive ability (reviewed in Bonte et al., 2012, Tigreros and Davidowitz, 2019).

Fitness consequences of dispersal have been shown in a variety of different traits and taxa and are broadly expected from life-history theory (Roff, 1992; Stearns, 1992). For example, longevity can be negatively correlated with dispersal (e.g., Zera and Brink, 2000, Khuhro et al., 2014). Changes in reproductive ability may however, be described by a diverse array of metrics of fecundity or reproductive effort, success or failure, including egg size and number laid, nutritional content, egg-to-adult viability or hatching rates. These, in turn, may be related to evolutionary fitness in complex ways and, consequently, it is often un-

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clear what the most relevant metric is by which to measure such trade-offs (Zhao and Zera, 2001; Zera and Zhao, 2006; Zhao and Zera, 2006). Key to the assumption of trade-offs related to greater dispersal, flight or movement ability is that organisms have finite resources which must be allocated to reproduction, growth, metabolism and maintenance (Roff, 1992; Stearns, 1992). Flight-fecundity trade-offs are expected to incur either a biomechanical flight constraint cost, in which increased mass from e.g., carrying eggs, results in a greater wing loading and thus penalizes energetic reserves or incurs direct physiological costs (e.g., compromised immune function, antioxidant potential (Levin et al., 2017)). Alternatively, there may be adaptive negative correlations when switching from flight periods to egg production if conditions require a change of reproduction strategy, or due to adaptive positive correlations in which optimal flight and high fecundity are favoured to colonize habitats (Tigreros and Davidowitz, 2019).

The classic flight-fecundity trade-off involve several reports of insect species, including *Drosophila*, showing decreased fecundity due to increased distance travelled or increasing amount of time spent in induced, tethered flight (e.g., Roff, 1977) and has been investigated across roughly 50 monomorphic species of insects (for a review see Tigreros and Davidowitz, 2019) including butterflies (e.g., Karlsson and Johansson, 2008), crickets (e.g., Mole and Zera, 1993) and moths (e.g. Khuhro et al., 2014) using diverse methodological approaches. Of the 70 studies reviewed in Tigreros & Davidowitz (2019) only 9 showed a clear flight-fecundity trade-off. Most research to date focuses on wing-dimorphic species (Guerra, 2011; Tigreros and Davidowitz, 2019). Mole & Zera (1993), for example, reported how reproductive efficiency is sacrificed in long-winged female crickets that allocated energy to flight muscles and larger wings rather than into egg production, like their short-winged counterparts. This is largely owing to the fact that construction, maintenance and operation of flight muscles incurs substantial energetic costs (Reinhold, 1999; Marden, 2000). Increasingly, wing monomorphic species such as beetles (David et al., 2015) moths (Khuhro et al., 2014) and bees (Helm *et al.*, 2021) are becoming the focus of investigation, and has been systematically reviewed recently in Tigreros and Davidowitz (2019). Their review highlighted how studies of wing monomorphic species generally do not find consistent evidence of flight-fecundity trade-offs, although the choice of study methodology may influence the outcome of this comparison, perhaps suggesting that assessments based on wing-dimorphic species have biased the expectation in the research field to some extent towards strong trade-offs, yet there is limited systematic research across diverse taxa to draw any robust conclusions.

It is widely expected that there will be increasing reliance upon dispersal to offset or mitigate changing climate conditions. On the one hand this could be a consequence of enhanced dispersal ability leading to individuals securing new, less stressful, habitats, or alternatively, species with disperser phenotypes and genotypes will be more capable of achieving range shifts, especially if living in fragmented, patchy habitats (Pecl et al., 2017). Consequently, understanding movement ecology is critical to forecasting future species' distributions (Pecl et al., 2017). Moreover, insect population dynamics are likely to respond readily to climate change since development rates, metabolic rates and longevity are directly affected by temperature (e.g., Karlsson and Johansson, 2008, Irlich et al., 2009). Thus, flight-fecundity trade-offs could be central factors influencing how population dynamics translates into changes in species' geographic distributions, potentially affecting gene flow among populations. On the other hand, species that thrive readily after flight, movement or dispersal, or overcome, or are able to minimize, a flight-fecundity trade-off may be more capable of colonizing novel habitats and achieving higher fitness. Mixed evidence exists for this notion since dispersal-reproductive trade-offs have been shown to be absent in some invasive plant species (Lambrecht-McDowell and Radosevich, 2005) but pronounced in the native population of an invasive aphid (Zhang et al., 2008). However mixed evidence for trade-offs could be due to variation among studies in their methodology and ex-

perimental approach. For example, tethered flight under tightly controlled laboratory conditions would most likely produce an outcome different from what might be observed in the field (e.g., Terblanche, 2014; Steyn et al., 2022), but the source of variation would be unclear. Perhaps tethered flight induces stress that would contribute to inflating any apparent 'costs'. Thus, controlling for multiple factors and systematically dissecting their effects also enables insights into the potential sources of trait variation that might impact hypothesis testing. Moreover, in cases where such a trade-off exists, the duration or persistence of the trade-off across generations typically remains unclear (Zera and Brink, 2000) but is nonetheless important for understanding the evolutionary significance thereof.

Here, we therefore aimed to experimentally estimate the prevalence, magnitude and direction of flight-reproduction trade-offs between flies that choose to fly (referred to as 'dispersers' hereafter) and those that do not ('residents' hereafter) under semi-natural, field 'cage' conditions. We also sought to 1) replicate these findings across multiple species to better determine if the outcomes were species-specific, and 2) to explicitly control for a host of potential confounding effects that are typically overlooked (including maternal effects, age, recent weather conditions and nutritional history), to better understand the context-dependencies of any such patterns. Individually, each of these factors has well-documented impacts on fly reproduction under laboratory conditions (review in e.g., Tigreros and Davidowitz, 2019). We also aimed to explicitly consider morphological covariates (body size, wing-loading) that might influence the outcome of any potential flight-reproduction or fitness trade-off (see e.g., Kingsolver, 1999), as these may be related to successful dispersal (or vice versa) and/or reproductive output. We do so using replicated, freshly-established isofemale lines of five *Drosophila* species collected in South Africa representing different climate ecotypes, measured under standard conditions and explicitly controlling for several well-known potential confounding effects (e.g., age, field effects) using replicated field tunnel (fine-mesh cage) releases.

Materials and methods

Collection and rearing of Drosophila *species*

Drosophilidae were sampled from six locations in the Western Cape and Kwa-Zulu Natal Provinces, South Africa (described in De Araujo et al., 2019a). Trapping consisted of buckets placed in shaded habitats, baited with ripe fruit or mushrooms in different combinations to better capture Drosophilidae diversity. Flies were collected by aspiration and transferred into plastic bottles filled with Bloomington's standard corn-meal diet medium (http://flystocks.bio.indiana.edu/Fly_Work/media-recipes/bloomfood.htm) to start isofemale lines from a minimum of 10 field-inseminated females per species. Species were identified using the universal cytochrome oxidase I (COI) primers (Folmer et al., 1994) and confirmed against GenBank databases. Five species were readily reared and positively identified (Table 1): *Drosophila busckii* Coquillett, 1901, *D. immigrans* Sturtevant, 1921, *D. simulans* Sturtevant, 1919, *D. ananassae* Doleschall, 1858 and *D. melanogaster* Meigen, 1830.

To eliminate maternal effects and field effects (e.g., acclimatization or recent thermal history), flies captured were reared under constant controlled conditions in the laboratory for at least two generations with *ad libitum* food availability until any experimental procedure was conducted. Flies were either reared at 23 °C (*Drosophila busckii* Coquillett, 1901, *D. immigrans* Sturtevant, 1921 and *D. simulans* Sturtevant, 1919) or 24–25 °C (*Drosophila ananassae* Doleschall, 1858 and *D. melanogaster* Meigen, 1830) to better reflect optimal growth temperatures for species from the main environment's sampled (Table 1, De Araujo et al., 2019a). Once flies started emerging (F1) they were transferred to a new bottle with fresh diet medium at standardized low density. This process was replicated until the F3 generation for all species, except *D. melanogaster* for which the F2 generation was used due to time constraints. All ex-

Table 1

The locations and description of collection sites, date of capture and bait type for the five Drosophilidae species captured and used in this study.

Species	Latitude (Decimal Degrees, °S)	Longitude (Decimal Degrees, °E)	Site description	Climate	Date of Capture (dd/mm/yy)
Drosophila ananassae	−29.7062	31.0444	Urban	Sub-tropical	22/03/18
Drosophila busckii	−33.8956	18.5642	Home garden	Mediterranean	02/11/17
Drosophila immigrans	−33.8956	18.5642	Home garden	Mediterranean	02/11/17
Drosophila melanogaster	−29.7013	31.1009	Coastal forest	Sub-tropical	08/06/18
Drosophila simulans	−33.8956	18.5642	Home garden	Mediterranean	02/11/17

periments were conducted using six-day old flies to minimize variation associated with aging and reproductive senescence (Bowler and Terblanche, 2008, Le Bourg, 2011, Colinet et al., 2015).

Flight performance

To test the flight effect (or simulated dispersal) in each species, a 20 m long tunnel (adapted from Kristensen et al., 2006, Hoffmann et al., 2007, Overgaard et al., 2010) was constructed and placed in full sunlight outdoors. The tunnel (20 × 1.5 × 1.5 m) was covered by a fine mesh material with a plastic window (40 × 30 cm) on both ends and sealed access points along the tunnel. Material dividers were sewn on at various points along the length of the tunnel (2 m, 10 m, 18 m) that could be closed, if desired, to prevent unwanted dispersal. In previous field experiments, distances ranging between 2 m and 30 m have been used to assess diverse phenotypic effects (e.g., Kristensen et al., 2006, a comprehensive literature review of methods and approaches employed to date in other flight-fecundity trade-off studies of insects is provided in Tigreros and Davidowitz (2019)) and given that some assays of *Drosophila* in our study took on average 2 h (or a maximum of 4 h) to disperse the length of the tunnel, and represent a substantial portion of the flies' reproductive lifespan, we consider our assays to be a fairly sustained flight event for *Drosophila* to detect potential flight-fecundity trade-offs. Furthermore, Roff (1977) demonstrated fecundity reductions in *Drosophila melanogaster* that flew tethered for 60 min alone. However, we argue that the tethered flight assays are short duration and intense, and obviously highly stressful for the fly. Further, these experimental conditions might be promoting reproductive trade-offs that do not occur in the field. We therefore explicitly aimed to have more realistic (semi-natural) field conditions in our experiment. While it may be deemed that conditions in our trials might not be that stressful compared to the tethered flight (laboratory) scenario, the nature of any trade-offs that might occur after these short distance, repeated 'hopping' flights made over a few hours in the wild nevertheless remains unclear.

Fly releases were only performed if the weather conditions on a given day met the following 3 criteria: 1) the air temperature was between 18 and 25 °C, 2) windspeed ≤3 m/s, and 3) it was a cloudless day (the latter to minimize potential variation in polarized light cue effects). Fly releases were replicated at least 3 times across different days using new flies each time. A bottle filled with 100 six-day-old virgin females (mixed from different isofemale lines) 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. At the start of the experiment, the bottle was opened and shaken gently to ensure that all flies left the bottle. Flies were then allowed to disperse freely in the tunnel while constantly being monitored by several observers evenly distributed along the length of the tunnel. From pilot trials and field observations, it was clear that walking was not a preferred locomotion method within the tunnel as no flies were observed consistently or even intermittently walking along the cage towards the bait-end of the tunnel. When female 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 material dividers in the tunnel were raised to prevent further dispersal; there were no observations of

flies flying back and forth between the start and end of the tunnel. A haphazard selection of 30 females that chose not to disperse were aspirated out of the 2 m starting segment of the tunnel and each fly was placed in a vial with a single virgin male to mate. The females that chose to fly to the end of the tunnel represent 'dispersers' and those that did not were classified as 'residents'.

Life history traits

Vials were then transported to the laboratory where each pair was placed inside a honey jar with three diet-medium-filled bottle caps as oviposition sites and checked at 20-hour intervals until the first batch of eggs had been laid. We think this egg batch would be significant for determining trade-offs as it represents a substantial portion of lifetime reproductive success and energetic investment. Once the first batch of eggs was observed, the males were removed and discarded and the females transferred to new vials with medium. This first batch of eggs was counted under a light microscope (Stemi 305, Zeiss, Germany) to measure reproductive effort. We proceeded to count only the eggs visible on the surface to prevent damaging the eggs and therefore more eggs could have been laid in the diet that could not be counted in a non-destructive way. Female survival was monitored daily until death to determine longevity and subsequently snap frozen and stored in a -80 °C freezer for morphological measurements. Bottle caps containing the first batch of eggs were placed in a diet medium-filled bottle and left to allow flies to complete their development and emerging adults were counted as a measure of reproductive fitness.

Morphometrics

For each female assessed for flight and reproduction, body mass and wing morphology were determined. Body mass was estimated by drying flies at 40 °C for 12 h after which flies were weighed on an ultra-microbalance (Mettler Toledo UMX-2) to 0.1 mg. Thereafter, one intact wing was carefully dissected and mounted on a slide with clear nail varnish. After dispersal and mating, some flies' wings were often damaged and in these cases we assumed wings were relatively symmetrical and left or right wings were used interchangeably. Wing area was estimated by making length and width measures taken between standard nodes across all wings of all flies and for each species using a calibrated Leica MZ-75 Automontage digital imaging microscope system. Wing loading was estimated as body mass (in mg) divided by a single wing's area x 2 (to yield the total wing area (in mm^2)).

Statistical analyses

As each release experiment consisted of a random subset of individuals pooled from each isofemale line at the tunnel release point, line identity was unknown for each recaptured fly. Thus, lines could not be distinguished or nested statistically within the dispersers and residents but we assume line was randomized sufficiently well as to not introduce any systematic biases. We used R version 3.3.3 (R Core Team, 2013) and 'lme4' (Bates et al., 2015) to perform mixed-effects analyses (*lmer* function) of the effect of dispersal category on traits of reproductive effort,

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. p-values were corrected for table-wide multiple tests using False Discovery Rates (q-value, Storey 2003). Significance is shown in bold.

Species	Trait	df	X^2	p value	q value
D. ananassae	Reproductive effort	1	0.009	0.924	0.990
	Reproductive fitness	1	0.138	0.710	0.990
	Time-to-first egg laying	1	1.146	0.284	0.852
D. busckii	Reproductive effort	1	1.969	0.160	0.852
	Reproductive fitness	1	0.028	0.866	0.990
	Time-to-first egg laying	1	0.000	0.996	0.996
D. immigrans	Reproductive effort	1	0.149	0.699	0.990
	Reproductive fitness	1	0.635	0.426	0.913
	Time-to-first egg laying	1	0.041	0.839	0.990
D. melanogaster	Reproductive effort	1	1.209	0.272	0.852
	Reproductive fitness	1	5.583	**0.018**	0.270
	Time-to-first egg laying	1	0.087	0.769	0.990
D. simulans	Reproductive effort	1	0.849	0.357	0.893
	Reproductive fitness	1	1.300	0.254	0.852
	Time-to-first egg laying	1	0.051	0.822	0.990

reproductive fitness or time to first egg laying for each species separately and also with all species pooled using a Poisson distribution and a log link function. 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 (lowest Akaike information criterion (AIC) scores). For all measures of reproductive output, the best models included replicate releases as a random effect. To report which factors were significant in each analysis, we systematically tested for the effect of disperser category (and species when pooled data were tested) and the interaction thereof, with likelihood ratio tests of the full model with the effect of interest against a model without the effect of interest, to obtain a p-value. Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. To evaluate longevity between dispersal categories, a Cox-proportional hazards model was run in R ('survival' package, Therneau and Grambsch 2000) and a probit model in SAS to generate estimates of lethal time where 50% of the individuals in a population had died and compared ±95% confidence limits. To determine whether wing loading influenced the flight ability (disperser category) of the different *Drosophila* species, a GLM with a binomial distribution and a logit link function with dispersal category (0 or 1) as the dependent variable and wing loading as the independent variable was run.

Results

In all traits examined, species differed significantly (reproductive effort: df=4, χ^2=185.53, p<0.001; reproductive fitness: df=4, χ^2=335.87, p<0.001; time-to-first egg laying: df=4, χ^2=17.946, p<0.001; Fig. 1) but across species the disperser category effect (df=1, χ^2=0.05, p=0.830) and its interactions were not significant (*lmer* model accounting for replicates, reproductive effort: df=646.14, t=0.723, p=0.470; reproductive fitness: df=642.30, t=0.079, p=0.937; time-to-first egg laying: df=646.30, t=0.358, p=0.721). Interestingly, although *D. busckii* took the longest to lay eggs (Fig. 1C), it laid the most eggs (Fig. 1A) and had the highest number of viable offspring (i.e., high reproductive effort and fitness) (Fig. 1B). *Drosophila simulans* took the shortest time to lay eggs (Fig. 1C), and *D. ananassae* performed the poorest by laying the least number of eggs (Fig. 1A) and produced the lowest number of adults overall (Fig. 1B). There was no significant difference between disperser and resident flies for the trait of reproductive fitness (Table 2). While both *D. melanogaster* and *D. busckii* laid apparently fewer eggs post-dispersal than resident flies, when accounting for experimental replicates in the mixed-effect models, this was not significantly different (Table 2; Fig. 1). Although longevity varied between species

Table 3

Results of generalized linear models investigating the wing loading between dispersal groups (disperser or resident) in five different *Drosophila* species.

Species	Trait	df	z value	p value
D. ananassae	Wing loading	33	0.547	0.584
D. busckii	Wing loading	33	1.308	0.191
D. immigrans	Wing loading	17	−0.349	0.727
D. melanogaster	Wing loading	63	0.133	0.894
D. simulans	Wing loading	87	2.487	**0.013**

(df=4, χ^2=106.735, p<0.0001), the disperser category effect was not significant (df=1, χ^2=0.428, p=0.513; Fig. 1D).

In all species, except *D. simulans* (df=87, z=2.487, p=0.013), wing loading did not affect the disperser category (resident or disperser) (Table 3; Figs. 2 and 3) of flies. In *D. simulans*, dispersers had higher wing loading compared to residents.

Discussion

Here we systematically investigated whether a flight-reproduction trade-off is present in any of five *Drosophila* species using a standardized approach in replicated, semi-natural releases in a field setting. We explicitly controlled for a host of potential confounding effects that might impact fitness or reproduction estimates, including recent seasonal or nutritional history, age and parental effects. These are factors that are not typically well accounted for in studies of dispersal or movement ecology, especially those that focus on understanding flight-fecundity trade-offs at the species or population level (Tigreros and Davidowitz, 2019) despite well-known effects thereof (e.g., Kingsolver, 1999). Given that only one of the five species showed the expected trade-off for only one of the traits of reproductive fitness, the results of our experiments suggest that the classic flight-fecundity trade-offs are perhaps rarer than expected. For *D. melanogaster*, more adults emerged from the eggs laid by the residents compared to disperser flies, suggesting, at least in this species, that the flight-fecundity trade-off might well translate into an evolutionary fitness consequence, although this effect disappeared when controlling for false discovery rates across the table-wide test of the hypothesis (Table 2). This pattern was not however consistent across traits scored, nor was it widespread across the species investigated here, despite the diverse suite of fitness-related traits scored (see discussions in Malmqvist, 2000, Elkin and Reid, 2005, Hoffmann and Sgrò, 2018). Thus, the major result from our empirical estimates here, and the first demonstration of such in a field setting, is in keeping with the conclusions reached by a recent systematic review on the topic (Tigreros and Davidowitz, 2019) in which they suggested that the generality of the flight-fecundity trade-offs may have been overestimated.

While flight-fecundity trade-offs have been relatively well documented in a handful of wing-dimorphic insect species, the evidence thereof in wing-monomorphic insect species is far less prevalent (Zera and Brink, 2000; Elkin and Reid, 2005). Such dispersal-reproduction trade-offs have also been shown in some plant traits (e.g., seed dispersal) (Tabassum and Leishman, 2018). Studies of the flight-fecundity trade-off in wing-dimorphic insects have 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. In many cases, these studies do not assess the dispersal component but reasonably assume that the wing polymorphism would lead to variation in dispersal distance between morphs. The same pattern has also been shown in the wing-monomorphic African armyworm moth (*Spodoptera exempta*) (Gunn et al., 1989) and the beet armyworm (*Spodoptera exigua*) (Jiang et al., 2010). Early work on laboratory studies of individuals of *Drosophila melanogaster* (Roff, 1977) and *Drosophila subobscura* (Inglesfield and Begon, 1983) found that forced flight (flies were at-
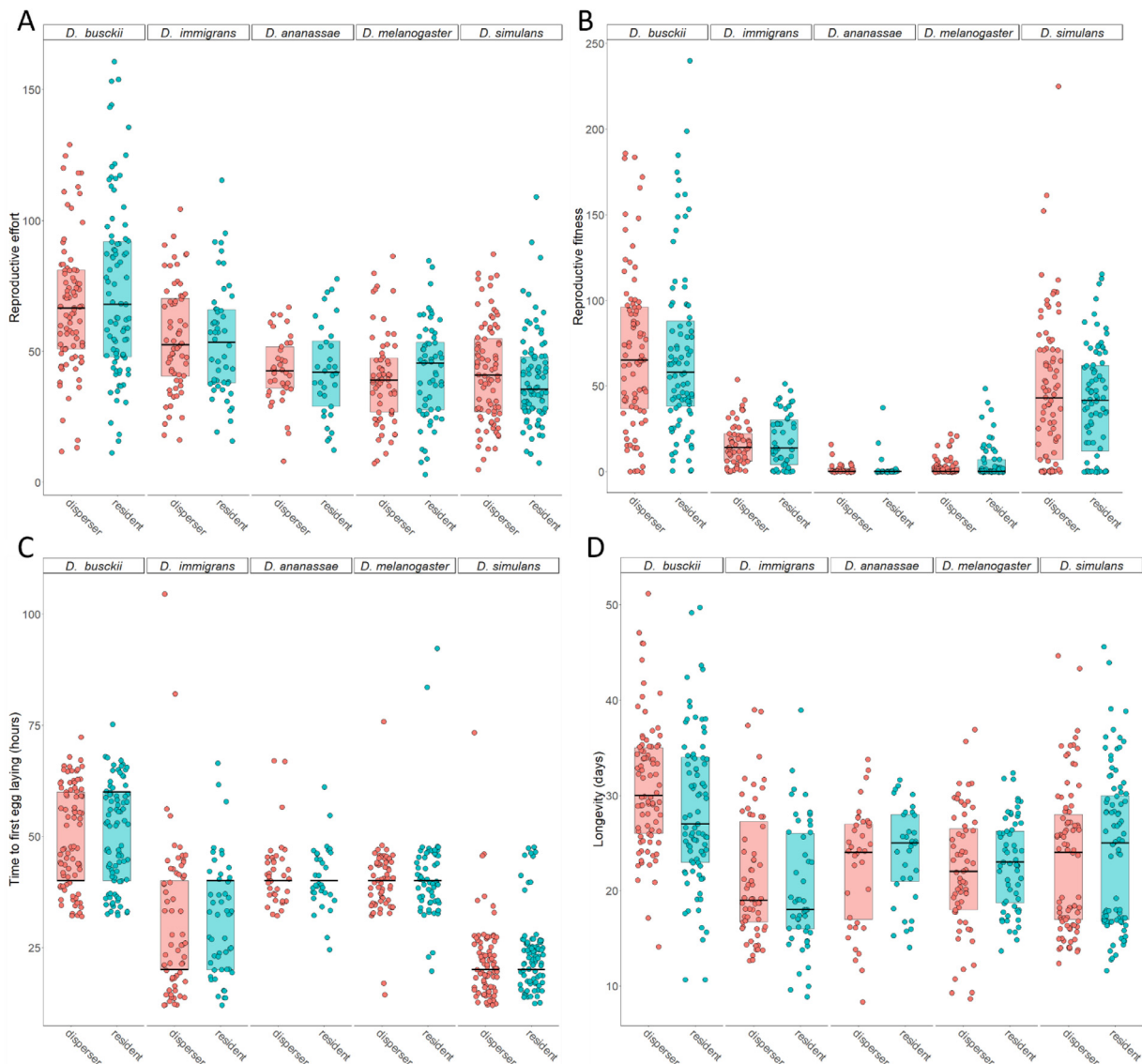
Fig. 1. Summary results (showing median, upper and lower quantiles, maximum and minimum values (whiskers) with raw data overlaid) for reproductive effort (A), reproductive fitness (B), time to first egg-laying (C) and longevity (D) for each *Drosophila* species categorized into disperser (red) and resident (green) flies. Columns within each panel represent the five species.

tached to a pin and stimulated to fly) could lead to lower subsequent egg production – an effect that even persisted for several days in some cases (e.g., Roff, 1977). Little information is available however, on the dispersal-reproduction trade-off in wing-monomorphic species more broadly (Tigreros and Davidowitz, 2019). Given how little evidence existed for such a trade-off in our study, it seems that the presence, magnitude and direction of the flight-fecundity trade-offs are perhaps species-, method- or context-specific. Alternatively, the lack of the trade-off detected may be due to the context-dependent nature of the trade-offs that was not fully probed in our experimental design. For example, in mountain pine beetles (Elkin and Reid, 2005), the abundance of food after dispersal lead to no decrease in reproductive ability after the beetles dispersed up to 50 m. Thus, high nutrient availability post-dispersal could override an energetic trade-off as the disperser drosophilids in our experiments were provided with food *ad libitum* upon collection at the end of the tunnel in the dispersal trial. Indeed, sustained flight, coupled with nutritional deprivation, induced fecundity trade-offs in *Bactrocera oleae* (Wang et al., 2009). Given the duration of our trials (maximum 1-2 h) and the relatively longer hunger cycles in *Drosophila* (>several hours) it is unlikely that flies simply moved because they were hungry in our

assays. Indeed, it would be of further interest to restrict food resources or nutritional status of flies, or assess multiple interactive stressors more generally, on the outcome of tests of the flight-fecundity trade-off hypothesis in *Drosophila*. Boldness and general behavioural propensity may well be contributing to tunnel dispersal in our trials and it would be useful to explore the drivers of these exploratory behaviours under artificial and field conditions in future, but this was beyond the scope of the present study. Even if behavioural propensity is a driver of any in-field tunnel dispersal, we expect the costs of dispersal to manifest in some tangible way, such as with measurably reduced reproductive output. As such, we were primarily interested here in realized dispersal. The major expectation is that dispersers will incur reproduction-related fitness costs, while those that do not disperse will not experience such costs. This notion is largely supported by results from studies using wing dimorphic species however any difference in reproductive output in those study systems may have been inflated by genetic and behavioural correlations associated with the wing morphs that have little to do with in situ dispersal costs.

Dispersal appears to have little effect on several fitness-related traits for the five diverse field-collected, laboratory-reared *Drosophila* species
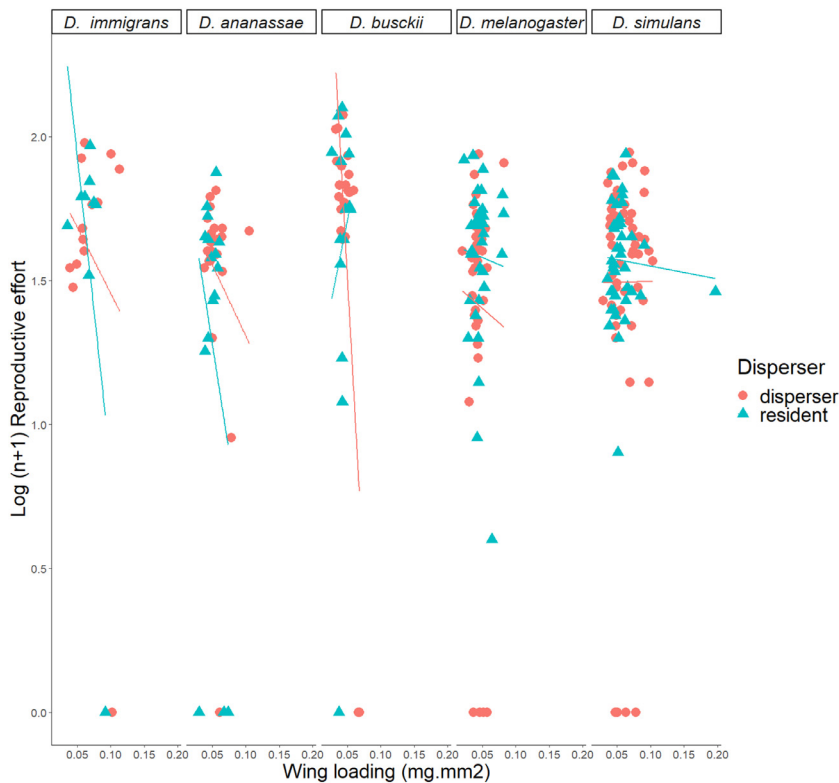
Fig. 2. Scatter plots of reproductive effort ($\log_{10} n + 1$) against wing loading (mg/mm^2) for each species, showing disperser and resident flies and the linear regression line fitted for each group.
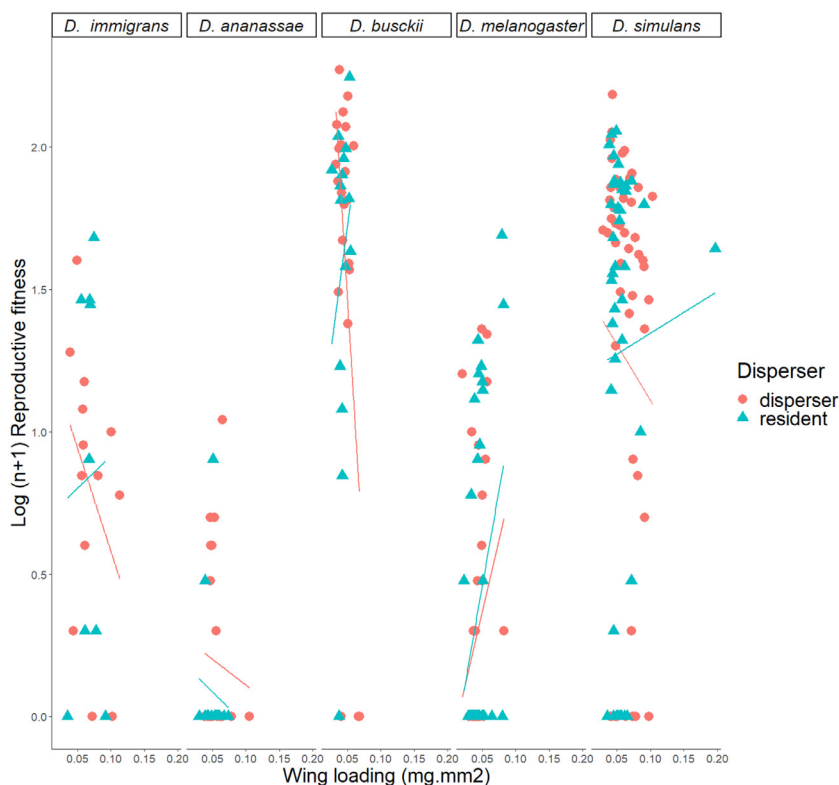

Fig. 3. Scatter plots of reproductive fitness ($\log_{10} n + 1$) against wing loading ($\log_{10}$ mg/mm^2) for each species, showing disperser and resident flies and the linear regression line fitted for each group.

examined here. There are several possible explanations for the neutral result obtained here. On the one hand, perhaps this suggests that *Drosophila* are well adapted to disperse and reproduce. Comprehensive, standardized assessments across other insect taxa could help resolve this possibility. Alternatively, costs might well exist but were not detectable under the conditions we employed, and future tests might need to consider subjecting flies to additional stressors (e.g., nutrient restriction) during or after dispersal. Helm et al. (2021) showed that nutritional sta-

tus influenced wing loading in *Osmia lignaria*, it is not clear however whether it impacts on flight performance. Moreover, it would be interesting to assess reproductive output if we could pre-screen flies for markers of dispersal and thus include disperser prone flies that have not previously flown as a control or reference group. Alternatively, one could perhaps use a gene-editing approach to artificially modify or eliminate flight once we know more about the mechanistic basis of dispersal. Given the limited support for flight-reproduction trade-offs that

we found here, we propose that the magnitude of these trade-offs, and their variation among species is unlikely to aid insect invasion success, or be more generally associated with niche breadth (De Araujo et al., 2019b), rapid colonisation potential, or expanding geographic distributions. Tests across a range of more niche-specialist species would be necessary to validate this idea further. Consequently, we argue that flight or dispersal events occurring at the temporal and spatial scales measured here, and any associated effect thereof on reproductive output or fitness, is unlikely to substantially enhance the ability of a particular *Drosophila* species to be successful colonisers of novel habitats. Consequently, based on the present results we argue that dispersal is either cheaper than expected, or the costs manifest differently than typically expected. We propose lost opportunities (i.e., time for other behaviours, such as mate-finding, mating or foraging) could pose substantial fitness cost to dispersal that are typically poorly explored. Future studies should attempt to differentiate among such costs in wing monomorphic species and could also explore sampling species at various distances from the release point in the tunnel to characterize the potential genetic differentiation between dispersing flies.

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Data accessibility

Raw data will be made available on a public repository upon acceptance of the article.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

John Terblanche is on the editorial board of the journal but is not involved in any way in the peer-review process and does not have access to anonymous referee information.

CRediT authorship contribution statement

Liana I. De Araujo: Funding acquisition, Methodology, Data curation, Formal analysis, Writing – original draft. **Minette Karsten:** Conceptualization, Funding acquisition, Resources, Formal analysis, Writing – original draft, Supervision. **John S. Terblanche:** Conceptualization, Funding acquisition, Resources, Formal analysis, Writing – original draft, Supervision.

Data availability

The source data from this study are provided as supporting information.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cris.2023.100060.

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