ORIGINAL RESEARCH

Morphological shifts in response to spatial sorting of dispersal behaviour in red flour beetles across multiple generations

P. A. Arnold[1,2] (iD), P. Cassey[3] & C. R. White[1,4]

[1]School of Biological Sciences, The University of Queensland, Brisbane, QLD, Australia

[2]Division of Ecology and Evolution, Research School of Biology, The Australian National University, Canberra, ACT, Australia

[3]School of Biological Sciences, The University of Adelaide, Adelaide, SA, Australia

[4]Centre for Geometric Biology, School of Biological Sciences, Monash University, Melbourne, VIC, Australia

Abstract

Dispersal is a key component of life history that influences individual fitness, population dynamics and range expansion. Morphological traits that are functionally related to dispersal may change through the spatial sorting of phenotypes at population range edges and invasion fronts. Generally, since larger invertebrates are expected to have greater dispersal capacity, larger body size and mass is expected to evolve in dispersers. Yet, different study systems exhibit a range of trait-specific and sex-specific responses to spatial sorting. Furthermore, a persistent issue in this field is that spatial sorting can be challenging to separate from natural selection and population dynamics. To address this, here we investigated the responses of dispersal and morphological traits to simulated spatial sorting into disperser and non-disperser red flour beetles (*Tribolium castaneum*) using a controlled laboratory dispersal system that isolated spatial sorting from natural selection and allowed sex-specific dispersal traits to evolve independently. After seven generations of spatial sorting, the time taken to disperse decreased in dispersers and increased in non-dispersers, with males dispersing more quickly than females. In contrast to expectations that dispersers would increase in body mass and size, we found the opposite. Body mass and morphology diverged such that dispersers became smaller and narrower, while non-dispersers became larger relative to the randomly selected controls, but femur length did not change. The trait responses across generations were also sex specific. Divergence between male dispersers and non-dispersers was more substantial than females, both in dispersal and in some morphological traits. We hypothesize that small individuals were more biomechanically efficient at dispersing through the dispersal apparatus and were therefore the ones that dispersed more readily. We suggest that key differences in dispersal modes across biological systems may impact the morphological and phenotypic trait changes from spatial sorting processes in range expansions and biological invasions.

Introduction

Dispersal can be defined as any movement or behaviour with potential consequences for spatial gene flow, which is typically a process of departure from the local environment, movement through a potentially hostile matrix and settlement in a new habitat (Clobert et al., 2012; Ronce, 2007). During dispersal and range expansion, individuals at the expanding edge or invasion front of a population are subjected to strong selective pressures from novel environments and changes to population density, which can rapidly alter phenotypic traits (Shine et al., 2011). As such, individuals at range edges may express different morphological, physiological and behavioural traits that facilitate effective dispersal. For example, highly dispersive individuals (dispersers) have been found to have longer legs relative to body size (Phillips et al., 2006), larger body size (Laparie et al., 2013; Yarwood et al., 2021) and larger flight muscles (Heidinger et al., 2018) than less dispersive individuals (non-dispersers). Dispersers are a non-random sample of phenotypes in a population that tends to accumulate at the range edge, and they are constrained to mate with other highly dispersive individuals by proximity (Shine et al., 2011; Travis

et al., 2012). Therefore, reduced genetic heterogeneity at edges is expected, but the evolution of dispersal-related traits such as morphology may accelerate expansion rate until it is attenuated by inbreeding depression or abiotic factors (Perkins et al., 2013).

The evolutionary pressure at expanding edges is a combination of natural selection and spatial sorting. Recent theoretical models have demonstrated that spatial sorting as a process is analogous to selection acting through space rather than time, where variation in dispersal ability will sort individuals spatially (Peischl & Gilbert, 2019; Phillips & Perkins, 2019). Spatial sorting may be a transient process in natural dispersal, where dispersal rates and associated dispersal-related traits increase during range expansion, thereby accelerating expansion and potentially rescuing populations from deleterious mutations that can accumulate at range edges (Peischl & Gilbert, 2019). Isolating the effects of spatial sorting and natural selection is a significant challenge in natural populations or under field conditions, as both processes are acting simultaneously (Phillips & Perkins, 2019). Nonetheless, growing evidence suggests that spatial sorting of disperser phenotypes in previous and contemporary range expansion has driven rapid directional evolution of traits across many taxa (Hargreaves & Eckert, 2014; Laparie et al., 2013; Shine et al., 2011; Yarwood et al., 2021). Recent experimental mesocosm and laboratory studies of spatial sorting in insects have shown that rapid evolution of dispersal rates and demographic traits is possible (Ochocki & Miller, 2017; Renault et al., 2018; Tung et al., 2018; Weiss-Lehman et al., 2017). Such experiments offer opportunities to explore spatial sorting effects on dispersal-related traits in both sexes while controlling for factors such as environmental heterogeneity, population size and natural selection.

Overall, larger invertebrates are expected to have enhanced dispersal capacity thus body size is usually hypothesized to increase with range expansion (Renault, 2020). There is also strong evidence from field studies that phenotypic trait responses to spatial sorting can be sex-specific, where differences in resource allocation, ecological roles, fitness landscapes and physiology between the sexes can explain some of these differences (Breuker et al., 2007; Cote et al., 2017; Ducatez et al., 2012). Sex-specific dispersal behaviour can arise depending on many factors, including mate and resource availability, and the biased dispersal of either sex can also alter sex ratios and mate availability (Trochet et al., 2016). Theoretical models predict that in natural conditions, dispersal should evolve to converge between the sexes through feedbacks on mate availability (Meier et al., 2011). Separating the effects of spatial sorting from sex, by allowing each sex to evolve dispersal and dispersal-related traits independently, could provide insight into these dynamics.

There are several examples of morphological changes due to spatial sorting or dispersal behaviour, many of which have noted sex-specific changes. In an invasive ground beetle species (*Merizodus soledadinus*), elytron length, femur length, pronotum length and width, and body mass all increased as residence time at the location decreased (i.e. invasion-front individuals were larger) (Laparie et al., 2013). In another ground beetle species

(*Carabus hortensis*), pronotum width and body condition were increased at the expansion front (Yarwood et al., 2021), but notably, these changes were only significant in males. Body size in the kudzu bug (*Megacopta cribraria*) increased with proximity to the invasion front in a similar manner for both sexes in one study (Lovejoy & Lozier, 2021), but pronotum width decreased with proximity to the invasion front in a different study, which was largely explained by sex differences (Merwin, 2019). The body mass of an organism and the size of their relevant morphology to support locomotion (e.g. thorax muscles, wings or legs) can be strong predictors of dispersal ability (Arnold, Cassey, & White, 2017; Ducatez et al., 2012; Laparie et al., 2013; Phillips et al., 2006). These relationships may reflect a biomechanical association between morphology and movement performance, for example where larger individuals or those with longer limbs will inherently have longer stride lengths or improved locomotor ability (Losos, 1990; Matsumura & Miyatake, 2019; Phillips et al., 2006). Therefore, moving greater distances in shorter time periods with lower energetic investment may reduce the significant time and energy costs associated with dispersal (Bonte et al., 2012). The diversity of responses across these examples (largely field studies) highlights the need for further exploration of sex-specific morphological trait responses to spatial sorting in the absence of landscape and abiotic heterogeneity and other conflated ecological processes.

Red flour beetles (*Tribolium castaneum*), an invasive cosmopolitan pest beetle species, are an ideal model system for studying dispersal and population dynamics. Although *T. castaneum* can fly to disperse across a landscape (Daglish et al., 2017), they generally do not disperse great distances and do so relatively infrequently (Ridley et al., 2011). Rather, their primary modes of locomotion for dispersal (as defined earlier) are typically walking and climbing (Campbell, 2012; Cline & Highland, 1976). Laboratory apparatuses to simulate dispersal have been used in studies of *Tribolium* species for many decades (Arnold, Rafter, et al., 2017; Łomnicki, 2006; Matsumura & Miyatake, 2015, 2018, 2019; Morrison III et al., 2018; Naylor, 1959; Ogden, 1970a; Prus, 1963; Renault, 2020; Ziegler, 1976), and some have be used specifically to test spatial sorting theory (Melbourne & Hastings, 2009; Ochocki & Miller, 2017; Szűcs et al., 2017; Weiss-Lehman et al., 2017). Although male *Tribolium* sometimes disperse more rapidly than females (Ogden, 1970b; Prus, 1966), both sexes readily disperse (Arnold, Rafter, et al., 2017; Ziegler, 1976).

In the present study, we established a laboratory spatial sorting protocol to sort individuals as non-dispersers (philopatric or range-core) and dispersers (invasion front or range edge) using microcosm dispersal apparatuses (Arnold, Rafter, et al., 2017). We then quantified the effect of spatial sorting on a suite of morphological traits frequently associated with dispersal in invertebrates: body mass, pronotum length and width, elytron length and width, and femur length. We used simulated spatial sorting processes to select lines based on individuals dispersing quickly through the apparatus (dispersers) and for individuals remaining philopatric for the longest (non-dispersers). This approach allowed us to identify morphological traits that significantly diverged between dispersers and non-dispersers, and to test for sex differences, after seven

generations of spatial sorting in controlled environments. By selecting on dispersal each generation, this design isolates spatial sorting because the population dynamics in each line are controlled, such that natural selection on growth rate should be identical. Furthermore, our approach kept sexes separately, which allowed dispersal and its related morphological traits to change independently in males and females. We tested the following predictions: (1) Dispersal behaviour would change over generations in accordance with the direction of spatial sorting (i.e. individuals from disperser lines would disperse faster and non-dispersers would disperse less and/or slower after generations of spatial sorting); (2) males would disperse faster than females due to their more active dispersal driven by mate-seeking behaviour; (3) dispersers would increase body mass and size compared with non-dispersers to facility dispersal capacity; and (4) morphological responses to generations of spatial sorting would differ between sexes, due to fundamental, innate differences in the dispersal behaviour of the sexes. By removing environmental heterogeneity and testing for morphological changes driven by spatial sorting in isolation and with sexes kept separately, here we identify underlying differences in dispersal responses between the sexes and due to spatial sorting in controlled laboratory dispersal apparatuses.

Materials and methods

Study system

A laboratory population of *T. castaneum* (QTC4 (Bengston et al., 1999); Postharvest Grain Protection Team; Department of Agriculture, Fisheries and Forestry; Brisbane, QLD, Australia) was used to establish experimental lines. The stock population was maintained on 210 g of media (95% wholemeal stoneground wheat flour, 5% torula yeast) in a 1 L container. The same media was used throughout experiments in 70 mL dispersal apparatus containers. Animals and experiments were maintained at 29.5 ± 1°C and 40–60% relative humidity.

Eight founding groups of 50 beetles (200 male and 200 female in total) were collected and sexed as pupae (Halstead, 1963) from the stock population. Pupae that were newly pupated or close to eclosion were not used, to maintain a controlled age structure within and among cohorts (adult beetles were aged 13–15 days post-eclosion when they were phenotyped). Founding groups represent four replicate lines for each sex that were paired for mating and generating subsequent generations. Each of these groups were housed in a 70 mL container with 15 g of flour for 6 days after initial collection. Lines were maintained as single-sex throughout dispersal assessment to prevent breeding during dispersal, which improved the efficacy of selection and allowed for clearer comparisons of sex-specific responses in isolation.

Dispersal apparatus

The dispersal apparatus is described in detail in Arnold, Rafter, et al. (2017). Apparatuses consisted of three containers (*A*, *B* and *C*) connected by tubing inserted through the lids,

containing cotton twine allowing one-way movement by climbing (Fig. 1a). Containers were 70 mL, and the tubing was flexible silicone 185 mm in length (4 mm internal diameter), arched over a 165 mm distance between lids that contained a single looped strand of cotton twine. Containers *A* and *C* held 15 g of flour, and *B* had a layer of paper to facilitate grip but no flour, representing an unsuccessful intermediate dispersal state (i.e. a hostile matrix). In container *A*, twine rested on the flour surface allowing beetles to climb up and into the tubing; the twine terminated 15–25 mm above the paper in *B* so that beetles could not return to *A*, and similarly, from *B* to *C*. Dispersal of *T. castaneum* through this apparatus design was consistent and repeatable (Arnold, Rafter, et al., 2017).

Containers housing each group of 50 beetles were connected to their respective apparatus (as container *A*) when beetles reached 2–5 days post-eclosion. The position of each apparatus within the room was randomized at each generation. Once container *A* was connected to each apparatus, beetles could disperse for up to 4 days. Counts of individuals within the dispersal apparatuses (in tubing: *A–B* and *B–C*, in containers: *B* and *C*) were conducted at 0900 h and 1700 h daily, until criteria were met (see *Spatial sorting criteria and breeding design*). Container *C* was detached, tipped into a dish so that the beetles could be counted, before being refilled and reattached. The number of beetles in container *A* was estimated as the starting number of individuals (*n* = 50) minus the sum of beetles in all other containers and tubing, as to not disturb the individuals that had not yet dispersed. Mortality in the apparatus across all lines and throughout the experiment was negligible (<1%).

Spatial sorting criteria and breeding design

In generation 1, the eight founding lines were run through the dispersal apparatus and used to establish four replicate lines for each of the sexes across three treatments: control, non-dispersers and dispersers. Spatial sorting of individuals in generation 1 was different to all subsequent generations. Dispersal within generation 1 was monitored and ceased when approximately 15 individuals remained in container *A* and approximately 15 individuals reached container *C*. This ensured that 15 individuals could be classified as non-dispersers and dispersers, respectively, and the remaining 15–20 individuals were randomly sampled from the apparatus to establish control (randomly assorted) lines. From the 15 classified individuals, five were randomly sampled to be paired with five individuals from their matching replicate line of opposite sex. These individuals were transferred to a 70 mL container with 5 g of flour to copulate for 5 days. Following this period, the 10 individuals were placed on 3 g of fresh flour and allowed 5 days to oviposit, after which time the adults were removed to allow offspring to develop over 23 days before collection as pupae. From these pupae, 50 of each sex were randomly sampled to establish the next generation of each line, and this process was repeated for seven generations in total. In generations 2–7, beetles could disperse until the following criteria were met for each of the
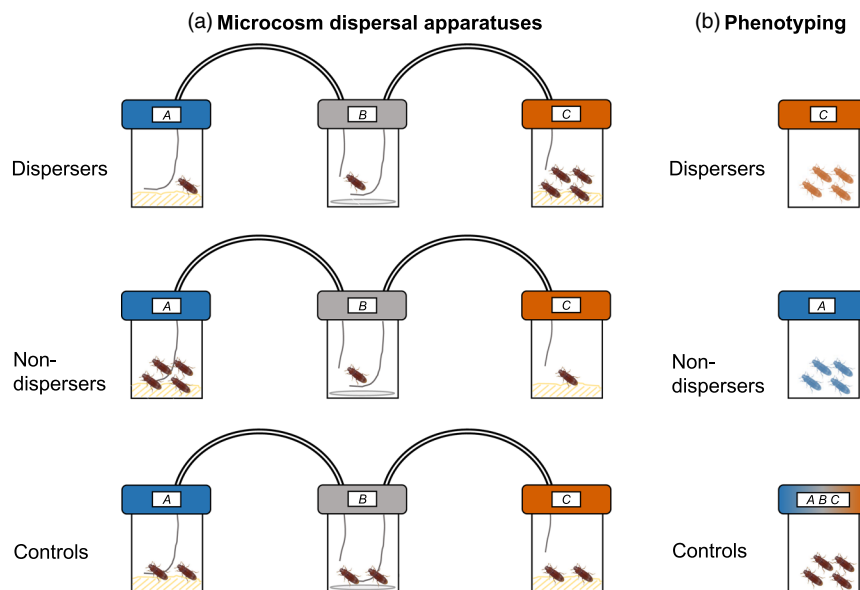
Figure 1 Microcosm dispersal apparatuses used to simulate spatial sorting of *Tribolium castaneum* adults into dispersers, non-dispersers and controls. (a) Dispersal apparatuses consist of three containers (patches) connected by tubing that contained string, allowing one-way movement from patch *A* to *C*. Patches *A* and *C* contained flour and patch *B* contained paper as a surface but no food resource (a hostile matrix). Colours represent the state of dispersal for individuals in each container (orange = dispersers, blue = non-dispersers and grey = intermediate, unsuccessful dispersal). (b) Individuals sampled from each line for phenotyping where beetle colours represent the container that the individuals were sourced from. After dispersal, individuals from disperser lines in patch *C* were measured, individuals from non-disperser lines in patch *A* were measured, and a mixed sample of individuals from control lines in all patches was measured.

assorted lines. For non-disperser lines, dispersal was ceased when 15–25 individuals had not yet emigrated from container *A*. If, after six counts (see *Dispersal apparatus*), the number of beetles remaining in container *A* exceeded 25, we sampled from these as non-dispersers. For disperser lines, dispersal was stopped when 15–25 individuals had successfully emigrated to container *C*. For control lines, dispersal was stopped after five counts; then, individuals were removed from the apparatus, mixed together, and were then randomly sampled from as controls. Up to 10 individuals from each line were phenotyped in generations 1 and 7 (Fig. 1b); therefore, sample sizes were $n = 36–40$ in each sex, dispersal and generation combination (Table S1).

Time taken to disperse and proportion of dispersers

Population-level rate of dispersal was assessed as the time taken for 15 beetles to successfully disperse to container *C* (h), matching the criteria for the breeding design as above. We controlled for population size effects on dispersal rate by running each dispersal apparatus from an initial population size of 50 in container *A*. In non-disperser lines, although only individuals remaining in container *A* were selected and phenotyped, the population-level dispersal rates of these lines were still quantified. The proportion of successful dispersers was calculated at each time count, where a binary score of '0' was assigned when <15 beetles had dispersed to container *C*, and

'1' where ≥15 beetles had dispersed to container *C*, again matching the criteria for the breeding design.

Body mass and morphometrics

Individuals were weighed for fresh mass on a precision microbalance (±0.01 mg, XS3DU; Mettler-Toledo, Columbus, OH, USA). Morphological measurements were taken by capturing dorsal, and ventral images using a microscope-mounted camera (PL-B686; PixeLINK, Ottawa, ON, Canada). Length and width of the elytron and the pronotum were measured from dorsal images, and length of the hind femur segment as a proxy of leg length was measured from ventral images using ImageJ software v1.46r (Schneider et al., 2012).

Statistical analyses

Data were analysed using the R software environment for statistical and graphical computing version 4.0.3 (R Core Team, 2020). Descriptive statistics of morphological traits are provided in Table S1. We statistically tested for change in dispersal and trait responses between generation 1 and generation 7. The spatial sorting was applied in parallel for males and females (i.e. sexes were kept separated); therefore, their responses were also analysed separately to reflect the physical separation during the experiment. For plotting proportions of dispersers across time, we fitted Bayesian generalized linear mixed effects regression models with binomial distribution and

logit link function using the *bayesglm* function from the R package *arm* (Gelman & Su, 2018). We fitted generalized linear mixed effects regression models using *brms* (Bürkner, 2018). All *brm* models were run using four chains, each with 10 000 iterations, 5000 of which were sampling, with adapt_delta = 0.99 and max_treedepth = 15 to ensure effective chain mixing. Significance was assessed when the 95% credible intervals (CI) around the posterior mean estimate of a predictor variable did not overlap zero, which is indicated in bold. All response traits were calculated as relative values to the respective paired control lines, to test whether the disperser and non-disperser lines diverged from respective controls lines where beetles were selected randomly from a dispersal apparatus. Predictor variables were dispersal (i.e. dispersers or non-dispersers), generation, and the interaction between dispersal and generation, with line replicate included as a random effect. The interaction term is of key interest, where a significant interaction term identifies that the dispersers and non-dispersers diverged in trait responses between generations 1 and 7 of spatial sorting on dispersal behaviour.

Results

Population-level dispersal responds to spatial sorting

Populations of beetles were spatially sorted based on their dispersal propensity. The time that 15 beetles took to disperse relative to the control (randomly chosen) lines differed significantly after seven generations (Fig. 2a,d). Initially, the male and female beetles in generation 1 dispersed at similar rates and all beetles did not disperse within the 80 h (Fig. 2b, e). By generation 7, both females and males moved through the dispersal apparatus significantly faster than in generation 1, and as was expected, dispersers were significantly faster than non-dispersers relative to the control populations (Fig. 2c,f; Table 1). The divergence in responses between dispersers and non-dispersers was far more compelling in males (Fig. 2f) than in females (Fig. 2c), though the disperser divergence in both sexes was significant considering the change from generations 1–7 (interaction terms in Table 1 and Fig. 2a,d).

Significant body mass and morphological changes differ between sexes

Overall, body mass and several morphological traits diverged such that dispersers became smaller and narrower, while non-dispersers became larger relative to the randomly selected controls across the seven generations of spatial sorting. Body mass responded strongly to spatial sorting on dispersal behaviour (Fig. 3a), resulting in clear divergence between dispersers and non-dispersers relative to the control group (significant dispersal × gen. 7 interaction terms, Table 2a). To compare, the relative change in body mass within a given sex between dispersers and non-dispersers from generation 1 to 7 was then calculated from raw mass (mg) values (rather than relative to control values). Over seven generations, body mass decreased

in dispersers in both males (−5.98%) and females (−2.17%), whereas body mass increased in non-dispersers in both males (+3.24%) and females (+4.32%). Changes in the absolute trait values are presented in Figure S1 and relative percentage changes in morphological traits are presented in Table S2. Male and female beetles had diverging responses in pronotum traits (Fig. 3b,c). In females, pronotum length and width differed significantly between generations 1 and 7 (Table 2b,c), but divergence between dispersers and non-dispersers was not clear (Fig. 3b,c), whereas in males, pronotum width diverged significantly (Table 2c; Fig. 3c). Elytron width diverged significantly between dispersers and non-dispersers in both males and females, as did elytron length in males but not females (Table 2d,e; Fig. 3d,e). Femur length only differed significantly between generations 1 and 7 in females, but it did not diverge between dispersers and non-dispersers, and therefore did not respond to spatial sorting (Table 2f; Fig. 3f).

Discussion

Rapid changes in phenotypic traits in response to selective pressures at range edges or invasion fronts have been predicted from differences observed among natural populations. The distribution of phenotypes within a population can be altered by spatial sorting, a spatial analogue of natural selection (Phillips & Perkins, 2019), of individuals along a continuum from non-dispersers to dispersers (Shine et al., 2011). We used a laboratory dispersal system to apply the isolated process of spatial sorting on both sexes individually, thereby excluding spatially autocorrelated abiotic variables while controlling for population size and dynamics. We confirmed our first two predictions that spatial sorting can stimulate changes to dispersal behaviour and morphological trait changes over seven generations, where males did indeed disperse faster than females. However, contrary to our third prediction that dispersers should increase in size, body mass and size appreciably increased in non-dispersers of both sexes over the seven generations, and somewhat decreased in dispersers. Yet, our fourth prediction that there should be sex-specific changes in morphology was confirmed, albeit not coupled with the expected increase in body size in dispersers. Rather, significant increases in body mass, pronotum and elytron lengths and widths were apparent in non-dispersers, with different responses between males and females. Here, we discuss the implications of these findings in the context of spatial sorting and microcosm dispersal studies.

Theory predicts that spatial sorting should accelerate range expansion by favouring individuals with life history and phenotypic traits that promote dispersal (Peischl & Gilbert, 2019; Phillips & Perkins, 2019). Experimental invertebrate systems in laboratory conditions have been used to study fundamental ecological and evolutionary dynamics that occur during spatial sorting of phenotypes through range expansions (Melbourne & Hastings, 2009; Ochocki & Miller, 2017; Szűcs et al., 2017; Weiss-Lehman et al., 2017). By spatial sorting *T. castaneum* in experimental landscapes across eight generations in the laboratory, Weiss-Lehman et al. (2017) found that the probability of dispersal increased in range-edge populations at low density. However, they also observed that the intrinsic population
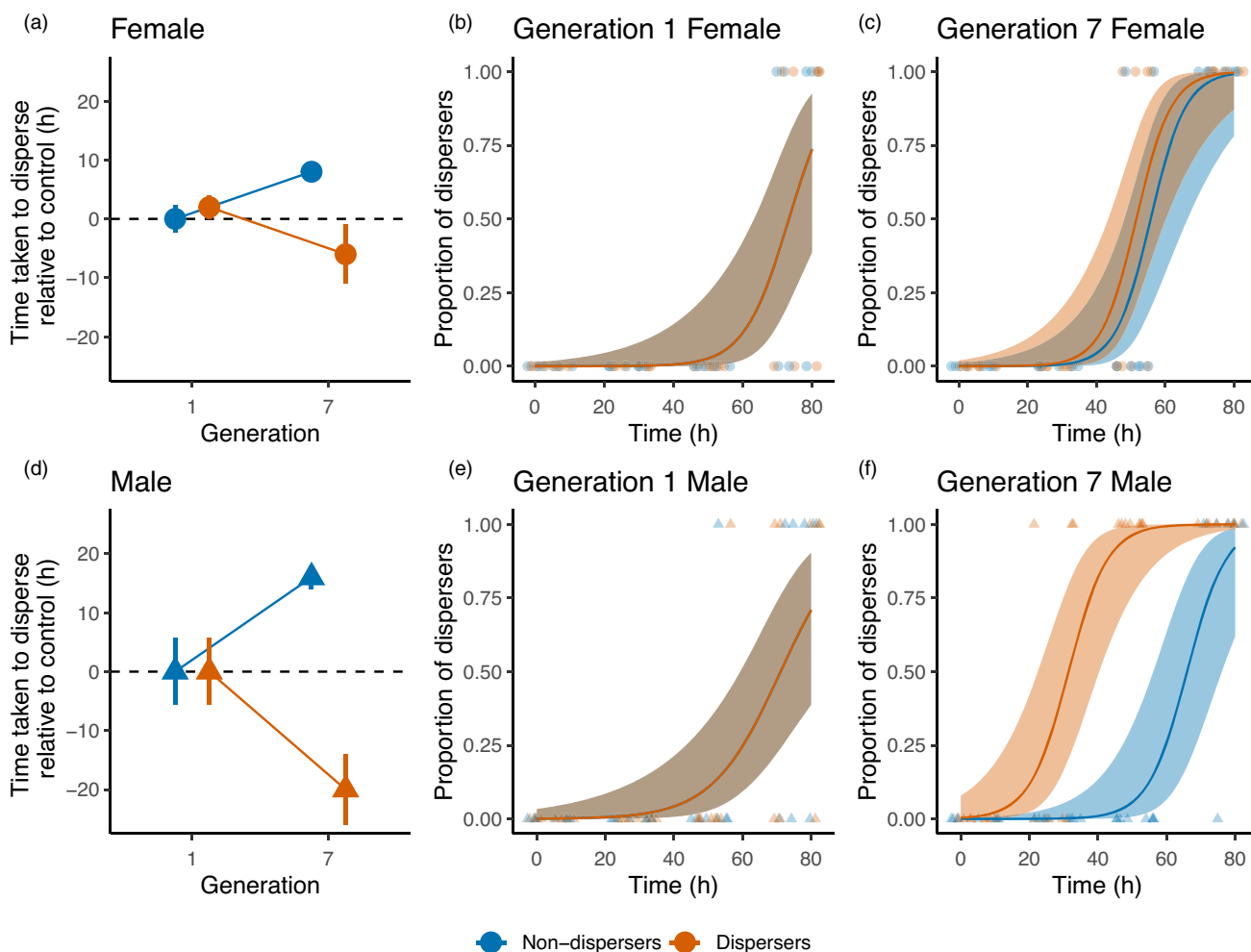
Figure 2 Population-level dispersal behaviour after spatial sorting in laboratory dispersal apparatuses. (a) Time taken for 15 beetles to disperse to container *C* in females in generations 1 and 7, where points represent means of four replicate lines, relative to their paired control lines. The proportion of replicate female disperser and non-disperser lines that had 15 beetles successfully dispersed to container *C* after 80 h in (b) generation 1 and (c) generation 7. (d) Time taken for 15 beetles to disperse to container *C* in males in generations 1 and 7. The proportion of replicate male disperser and non-disperser lines that had 15 beetles successfully dispersed to container *C* after 80 h in (e) generation 1 and (f) generation 7. Dispersers are coloured in orange non-dispersers in blue, and females are represented by circles and males by triangles. Error bars and shaded areas of fitted curves are both SE.

Table 1 Parameter estimates from model outputs for the relative time taken for 15 beetles to disperse between female and male *Tribolium castaneum*, comparing generations 1 and 7 after selection for and against dispersal

Time taken to disperse	Female		Male	
	Posterior mean	95% CI	Posterior mean	95% CI
Intercept (non-dispersers, Gen. 1)	0.345	(−6.154, 7.145)	0.352	(−11.610, 12.833)
Dispersal (dispersers)	1.997	(−7.610, 11.766)	−0.003	(−17.410, 17.702)
Gen. 7	**7.971**	**(0.206, 15.770)**	**15.937**	**(1.177, 30.993)**
Dispersal (dispersers) × Gen. 7	**−15.966**	**(−26.830, −5.147)**	**−35.875**	**(−56.846, −15.203)**

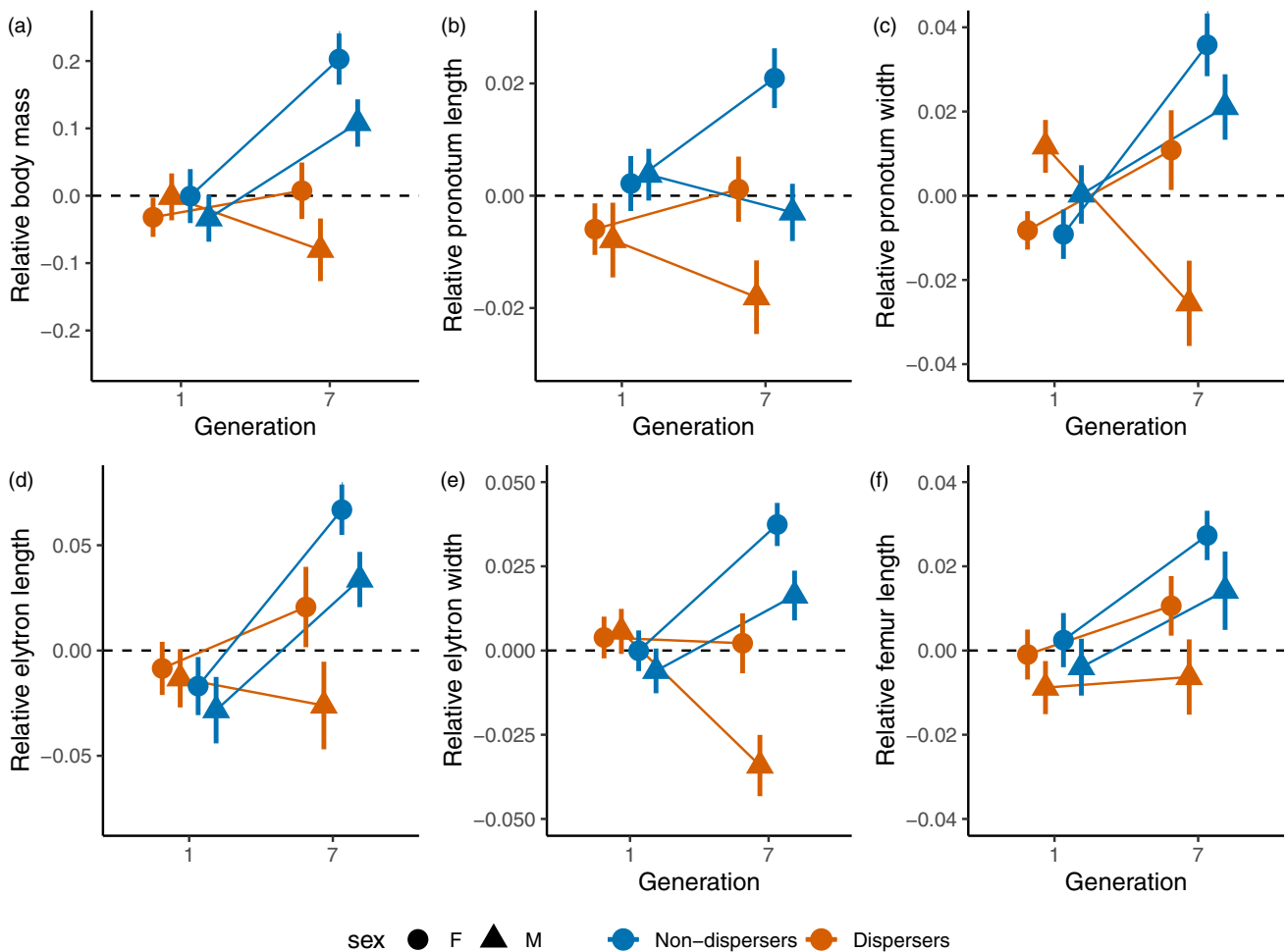Bold indicates that posterior means have 95% CI that do not overlap zero.

Figure 3 Morphological changes between generations 1 and 7 of spatial sorting for disperser and non-disperser red flour beetles, where all values are means ± SE calculated relative to mean trait values of paired randomly selected control lines. (a) Relative body mass, (b) relative pronotum length, (c) relative pronotum width, (d) relative elytron length, (e) relative elytron width and (f) relative femur length. Dispersers are coloured in orange and non-dispersers in blue, and females are represented by circles and males by triangles. Points have connected lines to highlight the direction of the morphological change and are offset within each generation to minimize point overlap and aid interpretation.

growth rate was lower in the edge compared with core or shuffled populations, but that the evolution of both traits was highly variable. Another study using *T. castaneum* that allowed or constrained evolution over six generations of experimental dispersal found that evolving populations grew faster and dispersed farther, which was density-dependent (Szűcs et al., 2017). In similar experiments using bean beetles (*Callosobruchus maculatus*), Ochocki and Miller (2017) found that 10 generations of spatial sorting significantly increased dispersal rate, which was highly variable among replicates. Collectively, these studies used experimental landscapes where dispersal mode is entirely walking between patches without any between-patch hostile matrix. In contrast, our study used multiple patches where dispersal among patches included vertical pathways to and through tubing (requiring both walking and climbing modes) and included a hostile matrix with potential failure during dispersal (Arnold, Rafter, et al., 2017). Our approach also isolated spatial sorting by controlling population

dynamics such that any selection on population growth rate and density effects should be identical among our replicate disperser and non-disperser lines. We note that we found greater relative changes in the non-dispersers, which suggests that the founding and control populations may have already been highly dispersive. Therefore, the responses of non-dispersers to spatial sorting may have been stronger than that of dispersers due to greater genetic variation in the direction of lower dispersal. We also explicitly allowed dispersal behaviour to change independently between the sexes. These key differences may partially explain why our study found that dispersal rate responded more strongly in males than females.

Both sexes of *T. castaneum* exhibit dispersal but differ in specific behaviours associated with reproduction (Arnaud & Haubruge, 1999; Arnold et al., 2016; Ziegler, 1976). The significantly higher dispersal rate of males in the single-sex dispersal apparatuses in our study is likely driven by mate-seeking behaviours, which occur earlier in males than females

Table 2 Parameter estimates from model outputs (a-f) for the relative morphological traits across seven generations of spatial sorting for and against dispersal and between sexes

	Female		Male	
	Posterior mean	95% CI	Posterior mean	95% CI
(a) Relative body mass				
Intercept (non-dispersers, Gen. 1)	−0.017	(−0.407, 0.208)	−0.041	(−0.232, 0.145)
Dispersal (dispersers)	−0.039	(−0.141, 0.062)	0.040	(−0.065, 0.147)
Gen. 7	**0.200**	**(0.101, 0.300)**	**0.148**	**(0.040, 0.256)**
Dispersal (dispersers) × Gen. 7	**−0.153**	**(−0.298, −0.014)**	**−0.227**	**(−0.375, −0.077)**
(b) Relative pronotum length				
Intercept (non-dispersers, Gen. 1)	0.002	(−0.020, 0.025)	0.003	(−0.017, 0.022)
Dispersal (dispersers)	−0.009	(−0.024, 0.005)	−0.011	(−0.028, 0.005)
Gen. 7	**0.019**	**(0.005, 0.033)**	−0.006	(−0.023, 0.010)
Dispersal (dispersers) × Gen. 7	−0.011	(−0.031, 0.009)	−0.004	(−0.027, 0.019)
(c) Relative pronotum width				
Intercept (non-dispersers, Gen. 1)	−0.009	(−0.046, 0.029)	−0.001	(−0.032, 0.033)
Dispersal (dispersers)	0.000	(−0.020, 0.020)	0.013	(−0.009, 0.035)
Gen. 7	**0.045**	**(0.026, 0.064)**	0.022	(−0.001, 0.044)
Dispersal (dispersers) × Gen. 7	−0.025	(−0.052, 0.002)	**−0.059**	**(−0.090, −0.028)**
(d) Relative elytron length				
Intercept (non-dispersers, Gen. 1)	−0.025	(−0.208, 0.042)	−0.032	(−0.127, 0.060)
Dispersal (dispersers)	0.007	(−0.034, 0.048)	0.019	(−0.026, 0.064)
Gen. 7	**0.083**	**(0.040, 0.123)**	**0.065**	**(0.020, 0.110)**
Dispersal (dispersers) × Gen. 7	−0.052	(−0.109, 0.004)	**−0.078**	**(−0.141, −0.016)**
(e) Relative elytron width				
Intercept (non-dispersers, Gen. 1)	−0.000	(−0.035, 0.033)	−0.007	(−0.038, 0.025)
Dispersal (dispersers)	0.003	(−0.016, 0.022)	0.013	(−0.009, 0.034)
Gen. 7	**0.037**	**(0.018, 0.056)**	**0.023**	**(0.001, 0.044)**
Dispersal (dispersers) × Gen. 7	**−0.038**	**(−0.065, −0.012)**	**−0.063**	**(−0.093, −0.033)**
(f) Relative femur length				
Intercept (non-dispersers, Gen. 1)	0.003	(−0.019, 0.024)	−0.005	(−0.035, 0.024)
Dispersal (dispersers)	−0.004	(−0.022, 0.015)	−0.004	(−0.026, 0.018)
Gen. 7	**0.025**	**(0.007, 0.042)**	0.019	(−0.003, 0.041)
Dispersal (dispersers) × Gen. 7	−0.013	(−0.038, 0.012)	−0.016	(−0.047, 0.014)

Bold indicates that posterior means have 95% CI that do not overlap zero.

(Ogden, 1970b; Prus, 1966), although females also disperse to lay eggs due to cannibalism of larvae and eggs (Flinn & Campbell, 2012). Pheromones and olfactory cues may play a role in dispersal since the presence of individuals in flour medium they inhabit can also 'condition' the medium by chemical secretions and frass accumulation over time, which can increase dispersal similarly to increasing density (Ogden, 1970b). Dispersers may have become more sensitive to pheromones, or these individuals could exhibit stronger mate-finding behaviours. Sex-specific dispersal behaviour is near universal, often male-biased in arthropods, due to different benefits and costs between the sexes (Trochet et al., 2016). By separating the sexes, here we found that dispersal changes in a sex-specific manner.

Spatial sorting and artificial selection on movement behaviour have resulted in changes to dispersal propensity and capacity across generations in *T. castaneum* (Matsumura & Miyatake, 2015, 2018; Weiss-Lehman et al., 2017) and *C. maculatus* (Ochocki & Miller, 2017). Studies on various species including cane toads (*Rhinella marina*), ground beetles (*Merizodus soledadinus* and *Carabus hortensis*) and eels (*Anguilla* spp.) have also found structural changes to body size and morphological traits when populations were spatially sorted (Hudson et al., 2016b,

2016; Laparie et al., 2013; Yarwood et al., 2021). Most of these field studies found that dispersers or those individuals closer to the range edge or expansion front were larger than non-dispersers (but see Merwin, 2019; Messager & Olden, 2019), in line with theoretical predictions (Renault, 2020). In contrast, we found that dispersers became smaller and non-dispersers larger over seven generations. Greater body mass is often associated with increased movement ability, endurance and energetic capacity to disperse (Forsman et al., 2011; Llewelyn et al., 2010; Renault, 2020), but may be unfavourable for certain types of dispersal.

One explanation for the divergence of body mass and size in opposing directions to our expectation is that spatial sorting could have also altered developmental rate or resource allocation during development. Our experimental design constrained the variance of pupal age and developmental stage at the time of collection to minimize differences in adult emergence time and to ensure that adults had matured prior to dispersal. Yet, since *T. castaneum* are holometabolous insects, the body size prior to pupation is indicative of adult size, hence those individuals that develop faster are also typically smaller (Arnaud et al., 2005; Davidowitz & Nijhout, 2004). Conversely, individuals that develop slower and allocate more resources into growth during

development would emerge larger, mature later and would therefore not disperse quickly. If movement behaviour in larvae reflects that of the adults, non-dispersers may have also increased their body size due to consuming relatively less energy by limiting movement compared with dispersers. To assess the contribution of developmental rate or resource allocation to body mass divergence, trait measurements would need to be made on earlier life stages. If dispersal rate is correlated with development rate, then development represents an underexplored aspect of spatial sorting and range expansion.

An alternative hypothesis for the body mass and size divergence is that small individuals are biomechanically and energetically better suited to dispersal by climbing than large individuals. For example, in *Anolis* lizards, selection pressure for increasing perch height led to significant shifts in morphology over 20 generations to facilitate better climbing (Stuart et al., 2014). Although body mass scales isometrically with energy stores and musculature (Lease & Wolf, 2011), it scales allometrically with metabolic rate (White, 2011), such that larger individuals have a lower resting and moving energy cost relative to their mass and energy stores (Reinhold, 1999). However, the absolute cost of transport is considerably lower for smaller animals (Halsey, 2016; White et al., 2016), and this difference is exacerbated when moving up an incline (Halsey & White, 2016). The work required to move a unit of body mass against gravity is the same irrespective of body mass (Halsey & White, 2016; Lipp et al., 2005); therefore, climbing is ultimately less costly for individuals with less mass to move. When climbing steep inclines, animals will also adjust their locomotor mechanics to reduce stride length and become more crouched (Birn-Jeffery & Higham, 2014). Smaller individuals can hold their centre of mass closer to the climbing surface and have an intrinsically shorter stride length than large individuals (Birn-Jeffery & Higham, 2014). Pronotum size can represent the room available for locomotor muscles and elytron size the amount of energy stores available in beetles (Laparie et al., 2013). However, the dispersal mode of climbing (particularly in our dispersal apparatuses) may be more limited by physical size than by energetics or muscularity.

In our study, males and females also differed in the slope and sometimes direction of their responses to spatial sorting, particularly the pronotum and elytron widths relative to the control population responses (Fig. 3) and in absolute terms between male and female dispersers (Figure S1). Although we cannot say definitively why these differences were observed, we generate hypotheses based on biomechanics of dispersal in the microcosm dispersal apparatuses. The overall lighter and smaller males also dispersed through the apparatus faster than did females, with male dispersers having reduced pronotum length, pronotum width and elytron width. These findings suggest that individuals with lighter and more narrow bodies were more biomechanically effective climbers that had the capacity to transport themselves more easily through the tubes between the patches in the dispersal apparatus. Hence, spatial sorting processes through these dispersal apparatuses may have partially filtered dispersal behaviour by size and shape.

We did not find any change in femur length between dispersers and non-dispersers. This contrasts with a study in *T. castaneum* that were artificially selected based on anti-predatory behaviour,

which found relatively longer legs evolved in both sexes that were selected for greater mobility (Matsumura & Miyatake, 2019). We hypothesize that this different finding is likely because our dispersing animals were required to climb, and animals with longer legs may not necessarily be better climbers. For example, invasion-front *R. marina* that have longer limbs relative to their body size exhibit increased rates of dispersal on the ground (Hudson et al., 2016; Phillips et al., 2006), but do not have enhanced climbing ability (Hudson et al., 2016a). Generally, animals that are efficient at climbing are not necessarily efficient when walking (Pontzer, 2016), and this decoupling between different dispersal modes (e.g. walking and climbing) may be an important difference among empirical studies on dispersal (Renault, 2020). Future studies on spatial sorting processes should also investigate whether different or mixed modes of dispersal, including flight, affect similar patterns of change to dispersal and dispersal-related traits and assess these at the individual-level as well as population-level.

Our experiment provides evidence that morphological traits can change considerably in response to spatial sorting on dispersal over just a few generations, altering the phenotypes of both dispersers and non-dispersers. The decrease in body mass and size of dispersers and notable increase in non-dispersers suggests that developmental processes and biomechanical efficiency may play key roles in determining which individuals have increased dispersal ability. The sex-specific responses to spatial sorting processes and potential involvement of development warrant further exploration into the energetics and allocation of resources across life stages and between the sexes of non-dispersers and dispersers.

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Author contributions

PAA, PC, and CRW conceived the ideas and designed methodology; PAA collected the data, analysed the data, and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Morphological changes between generations 1 and 7 of spatial sorting for disperser and non-disperser red flour beetles. Absolute trait values (means ± SE) for (a) body mass, (b) pronotum length, (c) pronotum width, (d) elytron length, (e) elytron width and (f) femur length. Dispersers are coloured in orange and non-dispersers in blue, and females are represented by circles and males by triangles. Points have connected lines to highlight the direction of the morphological change and are offset within each generation to minimize point overlap and aid interpretation.

Table S1. Summary of mean morphological trait values in each generation, dispersal and sex combination.

Table S2. Summary of relative change (%) in morphological traits from generations 1 to 7 in each dispersal and sex combination.