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RESEARCH ARTICLE

The distinct phenotypic signatures of dispersal and stress in an arthropod model: from physiology to life history

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ABSTRACT

Dispersing individuals are expected to encounter costs during transfer and in the novel environment, and may also have experienced stress in their natal patch. Given this, a non-random subset of the population should engage in dispersal and show divergent stress-related responses. This includes physiological shifts as expressed in the metabolome, which form a major part of responses to stress. We analyzed how metabolic profiles and life-history traits varied between dispersers and residents of the model two-spotted spider mite *Tetranychus urticae*, and whether and how these syndromes varied with exposure to a stressful new host plant (tomato). Regardless of the effect of host plant, we found a physiological dispersal syndrome where, relative to residents, dispersers were characterized by lower leaf consumption and a lower concentration of several amino acids, indicating a potential dispersal–foraging trade-off. As a possible consequence of this lower food intake, dispersers also laid smaller eggs. Responses to tomato were consistent with this plant being a stressor for *T. urticae*, including reduced fecundity and reduced feeding. Tomato-exposed mites laid larger eggs, which we interpret as a plastic response to food stress, increasing survival to maturity. Contrary to what was expected from the costs of dispersal and from previous meta-population level studies, there was no interaction between dispersal status and host plant for any of the examined traits, meaning stress impacts were equally incurred by residents and dispersers. We thus provide novel insights into the processes shaping dispersal and the feedbacks on ecological dynamics in spatially structured populations.

KEY WORDS: Amino acids, Biotic stress, Dispersal syndrome, Foraging, Metabolic profiling

INTRODUCTION

Dispersal, i.e. movement leading to gene flow, is a key trait at the nexus between ecological and evolutionary dynamics (Bonte and Dahirel, 2017; Clobert et al., 2012; Govaert et al., 2019). Not all individuals or populations show the same dispersal motivation and ability. The realization that this variation is often non-random has led to the study of dispersal syndromes, i.e. environmentally and/or genetically driven correlations between dispersal propensity/ability and other traits (life history, behavior, morphology, etc.; Ronce and Clobert, 2012). Dispersal syndromes are expected to influence the maintenance and distribution of phenotypic variation in space and time, as well as demographic dynamics or ecosystem functioning (Bonte and Dahirel, 2017; Cote et al., 2017; Massol et al., 2017; Ronce and Clobert, 2012). Among other things, dispersal syndromes may arise because dispersing and non-dispersing individuals actually experience different environmental and social contexts throughout their life, and thus different selective pressures favoring different trait combinations (Ronce and Clobert, 2012).

As their environment varies in space and time, organisms encounter a variety of conditions during their lifetime, and may be forced to deal with unfavorable or stressful conditions (Steinberg, 2012). A large body of research has shown that a mosaic of behavioral, physiological or biochemical responses are triggered during exposure to mildly to harshly stressful environmental conditions (Steinberg, 2012; Sulmon et al., 2015; Tuomainen and Candolin, 2011), with potential side effects on other individual life traits and fitness (Telonis-Scott et al., 2006). Individuals from the same population can show a large variation in stress resistance, which can be of both environmental (e.g. Henry et al., 2018; Ximénez-Embún et al., 2016) and genetic origin (Gerken et al., 2015; Rion and Kawecki, 2007; Rolandi et al., 2018).

Taking that into account, the relationship between dispersal and the ability of organisms to cope with environmental stressors is potentially complex. One could expect stress-sensitive individuals to increase their emigration rates under stress to limit the negative fitness consequences of stress; this would lead to a negative correlation between dispersal tendency and stress resistance. Such a negative correlation may also arise if stress resistance is costly. Indeed, costs incurred during dispersal (Bonte et al., 2012), in particular energetic and physiological costs, may limit dispersers' future stress resistance ability or vice versa (Matsumura and Miyatake, 2018). In all cases, however, the balance between fitness expectations at 'home' versus elsewhere will determine the eventual dispersal strategy. This means relationships between stress resistance and dispersal can be equally driven by fitness expectations after immigration in novel environments (Renault et al., 2018). In some cases, traits or contexts favoring dispersal do actually favor stress tolerance (Lustenhouwer et al., 2019). As the probability of arriving in, or crossing, marginal/suboptimal habitat should increase in heterogeneous environments, stress-resistant individuals may eventually profit most from dispersal, giving rise to positive correlations between these traits in such contexts.

The variation of physiological responses in relation to the environment and phenotype can be explored using metabolomic approaches (Bundy et al., 2008). As for morphological, behavioral and life-history traits (Beckman et al., 2018; Ronce and Clobert, 2012), information about whole-organism physiological state variation should help us understand the underlying mechanisms shaping dispersal variability. Metabolic profile changes in response to stress exposure, and their (putative) functional roles, have been extensively documented, especially in arthropods. Several amino

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acids (proline, alanine, glycine) or polyols (mannitol, sorbitol) are amassed when arthropods face saline, desiccation or thermal stress (Foucreau et al., 2012; Hidalgo et al., 2014; Teets and Denlinger, 2013). Starvation, i.e. the most severe form of food stress, affects the activity of several metabolic pathways, resulting in reductions of the quantities of essential amino acids as well as the use of energetic stores (Maity et al., 2012; Zhang et al., 2019). Tolerance to more limited food stress, e.g. resulting from low quality food owing to increased population density and metabolic wastes, has also been associated with polyol accumulations (Henry et al., 2018). In contrast, metabolite differences between dispersers and residents – even in interaction with the environment – are surprisingly understudied compared with other phenotypic differences (Cote et al., 2017; Ronce and Clobert, 2012). Among the few dispersal metabolomics studies published, van Petegem et al. (2016) found that more dispersive populations were characterized by lower concentrations in fructose, as well as lower activities of metabolic pathways associated with protein synthesis (as depicted by the lower amounts of a range of amino acids), the latter suggesting a reduced investment towards egg production in dispersive females. Meanwhile, Tung et al. (2018) reported an increased aerobic metabolism (higher amounts of glucose AMP and NAD) in flies selected for enhanced dispersal abilities. These differences may be explained by dispersers investing more than residents in dispersal-related traits to mitigate dispersal costs, which may lead them to have fewer resources to allocate to reproduction and to stress resistance mechanisms (Bonte et al., 2012), especially if dispersal and stress responses share metabolic pathways.

Using the phytophagous two-spotted spider mite, *Tetranychus urticae* Koch 1836, we combined metabolic profiling with more classical life-history and morphological measurements, and investigated whether and how dispersing and philopatric individuals differed from each other and in their resistance to a biotic stressor (Fig. 1). We expected dispersing and philopatric individuals to differ in several traits (i.e. to exhibit dispersal syndromes). More specifically, we expected dispersers to possess traits associated with lower competitive ability (Bonte et al., 2014), such as reduced body size or feeding. We also expected dispersers to show detectable evidence of incurred dispersal costs, such as lower fecundity (Matsumura and Miyatake, 2018). Finally, we expected to detect metabolic profile differences in line with these life-history and morphology differences (e.g. lower concentrations in amino acids; Van Petegem et al., 2016). *Tetranychus urticae* is highly generalist at the species level, with over 1100 documented host-plant species (Migeon et al., 2010). There is, however, strong among-population variation in the ability to survive and reproduce on specific host plants (reviewed in Rioja et al., 2017). In this species, metapopulation structures that favor the evolution of lower dispersal capacity or delayed dispersal also lead to the evolution

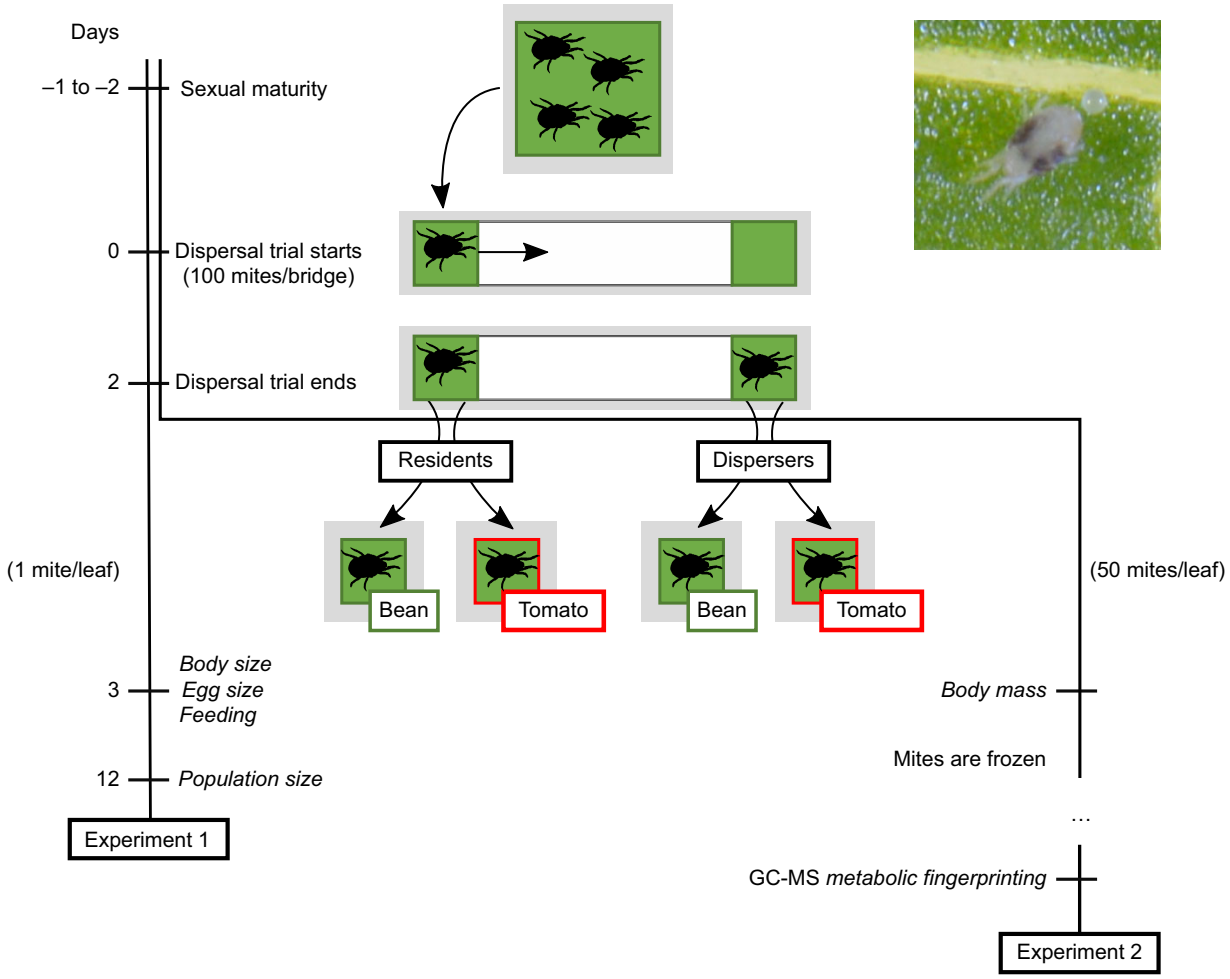

Fig. 1. Summary of the experimental design and timeline for the two experiments. Times are relative to the start of the dispersal trial, dispersal bridges were not all started at the same time. Phenotypic measurements are in italic; see Materials and Methods for details. Inset: an adult female *Tetranychus urticae* (body length: 440 μm) and a recently laid egg on a bean leaf (photo credit: Stefano Masier).

Journal of Experimental Biology

of higher tolerance to a new stressful host (De Roissart et al., 2015, 2016). In addition, dispersing mites that were forced to reintegrate densely populated patches had lower fitness than both residents and dispersers free to stay in low-density, presumably less stressful contexts (Bonte et al., 2014). Further, populations that had evolved higher dispersal also had lower amounts of some sugars and amino acids (De Roissart et al., 2016; Van Petegem et al., 2016), metabolites that are needed and potentially depleted when facing stressors. These converging lines of experimental results imply that dispersal and (biotic) stress resistance are negatively correlated, for instance because dispersers are stress-intolerant individuals that flee adverse conditions when they can, finding themselves at a fitness disadvantage if they cannot (owing to, for example, lower performance or ability to use resources, and/or individuals in lower body condition). Here, we more formally test this hypothesis at the individual level: although an overall performance decrease was expected in all stressed mites, we predicted that the metabolic and life-history profiles of dispersers would be more negatively influenced by exposure to the new stressful host plant than those of residents.

MATERIALS AND METHODS

Mite experimental population

We used mites from the bean-adapted LS-VL stock population, which has been maintained on whole bean plants since 2000 (*Phaseolus vulgaris* L. cv. Prélude) (De Roissart et al., 2016; Van Leeuwen et al., 2004). This experimental population harbors evolutionary meaningful amounts of standing genetic variation, as evidenced by experimental evolution and quantitative genetics studies (De Roissart et al., 2016; Van Petegem et al., 2018). Prior to experiments, synchronized mites of known age were obtained by allowing mites from the stock population lay eggs for 24 h on 7×7 cm² bean leaf squares (freshly cut from 2-week-old plants) set on wet cotton (50 mites per leaf square), then removing them. These eggs were then maintained at 30°C, 16 h:8 h light:dark until reaching adulthood, with the cotton kept hydrated. At this temperature, eggs took 3–4 days to hatch, and the offspring needed approximately 1 week to reach adulthood; adult longevity is approximately 10 more days. All adult mites used in subsequent experiments resulted from this procedure.

Dispersal trials

We used two-patch setups to sort dispersers from resident mites (Fig. 1, Fig. S1). Mated adult females 1 to 2 days post-maturity (the main dispersive stage; Krainacker and Carey, 1990) were randomly selected and placed on freshly cut 4 cm² bean leaf squares (start patches), which were then each connected to a 4 cm² empty bean leaf square (target patch) using a Parafilm bridge (2×8 cm²). Seventy bridges were used across all experiments. One hundred mites were initially placed on each start patch (25 mites cm^{-2}, an intermediate density in the range tested by Bitume et al., 2013). To keep leaves hydrated and limit mite escapes, bridges were set on wet cotton and external edges were covered with 2-mm-wide strips of moist tissue paper. Mites were then let free to disperse for 48 h at 25°C, 16 h:8 h light:dark; individuals found on the target patch were then deemed dispersers, and individuals found on the start patch were considered residents. Detailed records of disperser numbers were only kept for the first 10 out of 70 bridges. On these, 18.5% of mites dispersed to the second patch (95% confidence interval: 16.2–21.0%; range: 5–32%).

Experiment 1: Effects of dispersal status and host-plant quality on individual-level performance

Mites randomly sampled from available test patches (i.e. mated 3- to 4-day-old adult females) were placed individually on freshly cut 4 cm² leaf squares placed on wet cotton and surrounded by moist tissue paper (Fig. 1). Leaves were either stressful (4-week-old tomato, *Solanum lycopersicum* cv. Moneymaker) or suitable (bean). Forty replicates were made per dispersal status×host combination (N_{total}=160). We used population size (mites of all stages+eggs) after 10 days as a proxy of mite performance (Wybouw et al., 2015). Similar results were obtained using the number of eggs laid in the first 24 h as an alternative performance index (Fig. S2; correlation with 10-day population size, r=0.65). We evaluated feeding rate by taking pictures of leaves after 24 h (before any offspring of focal mites began to feed). Pictures were compared with those made at the start of the experiments, and the surface covered by feeding damage (chlorotic marks) was measured using ImageJ (Abramoff et al., 2004). We used the same images to measure average egg size and mite body length; two mites were not measured owing to bad positioning at the time of the photograph.

Experiment 2: Effects of dispersal status and host-plant quality on changes in metabolic profiles

We placed dispersers or residents randomly sampled from patches – 3- to 4-day-old mated females – in groups of 50 on fresh 4 cm² leaf squares prepared as above (Fig. 1). Between four and six replicates were made per dispersal×host treatment combination (N_{total}=20 samples). We left mites on leaves for 24 h, as preliminary tests showed that longer exposures to tomato at this density, as well as higher densities, led to high (>20%) mortality (densities of 50 or 100 mites per leaf were tested for up to 48 h). Mites were then collected in microtubes, weighed by group to the nearest 0.01 mg ($mass_{microtube+mites}$–$mass_{microtube}$), and snap-frozen in liquid nitrogen before storage at −20°C.

We then prepared samples for metabolomics analyses by gas chromatography–mass spectrometry (GC-MS), as described in Khodayari et al. (2013) and van Petegem et al. (2016). Samples were first homogenized into 300 µl of ice-cold methanol-chloroform (2:1, v:v) using a tungsten bead beating apparatus (RetschTM MM301; Retsch GmbH, Haan, Germany) at 25 Hz for 1.5 min. After addition of 200 µl of ice-cold ultrapure water, samples were centrifuged at 4000 ***g*** for 5 min at 4°C. An aliquot of 240 µl of the upper aqueous phase (containing polar metabolites) was then transferred to new chromatographic glass vials. Samples were vacuum-dried (Speed Vac Concentrator, MiVac, Genevac, Ipswich, UK), and resuspended in 30 µl of 20 mg l^{-1} methoxyamine hydrochloride (Sigma-Aldrich, St Louis, MO, USA) in pyridine, and kept under automatic orbital shaking at 40°C for 60 min of incubation. Finally, 30 µl of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA; Sigma-Aldrich, CAS number 25561-30-2) was added, and the derivatization was conducted at 40°C for 60 min under agitation. Prepared samples were then analyzed in a GC-MS system (Thermo Fisher Scientific, Waltham, MA, USA), using the same settings as in Khodayari et al. (2013) and van Petegem et al. (2016). The selective ion monitoring (SIM) mode was used to search for 60 primary metabolites common in arthropods and included in our spectral database (Van Petegem et al., 2016). Calibration curves were set up for each metabolite using pure reference compounds (1, 2, 5, 10, 20, 50, 100, 200, 500, 750, 1000 and 1500 µmol l^{-1}). Chromatograms were deconvoluted using XCalibur v2.0.7 (Thermo Fisher Scientific). Thirty-seven (out of 60) metabolites were successfully quantified in our samples and thus used in subsequent analyses (see Figs S3–S8 for full list). Concentrations were expressed in nmol mg^{-1} of mite fresh mass. Metabolites were grouped into six biochemical categories based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database hierarchy (Kanehisa et al., 2017): amines (N=3), free amino acids

(*N*=14), organic acids (*N*=5), polyols (*N*=7), sugars (carbohydrates excluding polyols, *N*=5) and other molecules (*N*=3, gluconolactone, glycerol-3-phosphate, phosphoric acid).

Ethical statement

The study complies with all relevant national and international ethical laws and guidelines. No ethical board recommendation was required to work on *T. urticae*.

Statistical analyses

We analyzed the effects of dispersal status, post-trial host and their interaction using trait-specific models, fitted with R version 3.5 (https://www.r-project.org/).

For the first experiment, we used a quasi-Poisson generalized linear model (GLM) for the number of mites at 10 days, and linear models for body size and egg volume (assuming spherical eggs). As 53.75% of mites did not feed, leaf consumption was analyzed using a binomial–lognormal hurdle model (see e.g. Fletcher et al., 2005), with feeding probability analyzed as a binary variable (fed/did not feed, binomial GLM) and leaf consumption in mites that did feed (*N*=60 on bean, 14 on tomato) using a Gaussian GLM with log link.

For the second experiment, we used a linear model to analyze mean fresh mass, and a permutational MANOVA (10,000 permutations) as implemented in the RRPP package (Collyer and Adams, 2018) to analyze the multivariate set of metabolite concentrations. A classical parametric MANOVA could not be used due to the high-dimensional structure of our dataset (number of molecules>number of samples) (Collyer and Adams, 2018). Concentrations were scaled to unit standard deviation before analysis to avoid metabolites with high baseline concentrations having a disproportionate influence on the result (van den Berg et al., 2006).

Then, to determine whether dispersal status or host plant altered the concentrations of specific categories of metabolites, we used the generalized linear mixed model approach proposed by Jamil et al. (2013) to analyze plant community data. Indeed, as a one-to-one correspondence can be drawn between the structure of community ecology and metabolomics datasets (sites=replicates, species=molecules, species traits=molecular characteristics), methods designed in one context can be readily applied to the other. We analyzed the concentration of all molecules grouped in a common univariate vector (37 molecules×20 replicates=740 concentration records) as a function of metabolite categories, sample dispersal status, host plant and all interactions. Furthermore, we included random effects of replicate (intercept-only) to account for the non-independence of molecular responses from the same sample, as well as random effects of individual metabolite to account for molecule-specific responses (random intercept as well as dispersal, host and dispersal×host effects). We fitted the model with the glmmADMB package (Fournier et al., 2012; https://rdrr.io/rforge/glmmADMB/), using the gamma family (as concentrations are always continuous and >0) and a log link. As the focus is on relative changes in concentrations, we used relative concentrations (mean concentration for all molecules set to 1) to avoid violations of model assumptions owing to a few molecules being present in much higher abundance than the others. We followed Schielzeth's (2010) guidelines to make model coefficients directly interpretable even in the presence of higher-order interactions: dispersal status and host were included in the model as centered dummy variables (resident or bean=−0.5, disperser or tomato=0.5). This and the log link lead to treatment×metabolite category model coefficients being directly interpretable as log(fold changes) in response to treatment. We estimated conditional and marginal R^2, i.e. the proportion of variance explained by model fixed effects (molecular category, treatment and interactions) and by the entire model (including random effects) respectively, following Nakagawa et al. (2017) and using the trigamma method. Plots were generated using the ggplot2 and cowplot packages (Wickham, 2016; https://cran.r-project.org/package=cowplot).

RESULTS

Experiment 1: Effects of dispersal status and host-plant quality on individual-level performance

Body size did not vary according to dispersal status, host plant or their interaction (ANOVA, mean±s.e.m. length=437±2 µm; Table 1). As expected from a putative stress treatment, mites exposed to tomato after dispersal trials performed worse than mites kept on bean (2.19±0.57 versus 48.71±7.69 individuals per leaf at 10 days; Table 1, Fig. 2), and were less likely to feed (Fig. 3, Table 1), with no significant effect of dispersal status or dispersal×host interaction in both cases (Table 1). Although feeding probability itself was not linked to dispersal status, dispersers that did attack their host plant fed less (26.99±10.62% less chlorotic damage) than residents (Fig. 3, Table 1).

Dispersers laid smaller eggs than residents (9.39±0.19 versus 10.04±0.19×10^5 µm^3), and mites exposed to tomato laid larger eggs

Table 1. Test statistics for the effect of dispersal status, host plant and their interaction on several *Tetranychus urticae* traits

Trait	Model	Number of replicates	Dispersal status	Post-trial host plant	Dispersal×Host interaction
Experiment 1					
Individual body size (length, µm)	Linear model	158	$F_{1,154}$=3.46; P=0.06	$F_{1,154}$=2.66; P=0.10	$F_{1,154}$=0.00; P=0.96
Total population size at 10 days	GLM (quasi Poisson)	160	X^2=2.32, d.f.=1, P=0.13	**X^2=81.97, d.f.=1, $P<2\times10^{-16}$**	X^2=0.13, d.f.=1, P=0.72
Feeding during the first 24 h					
Feeding probability	GLM (binomial)	160	X^2=0.15, d.f.=1, P=0.70	**X^2=56.79, d.f.=1, $P=4.86\times10^{-14}$**	X^2=0.12, d.f.=1, P=0.73
Feeding intensity (chlorotic damage, mm^2)	GLM (log-normal)	74	**X^2=4.83, d.f.=1, P=0.03**	**X^2=27.42, d.f.=1, $P=1.64\times10^{-7}$**	X^2=0.00, d.f.=1, P=0.97
Volume of average egg laid in the first 24 h (µm^3)	Linear model	136	**$F_{1,132}$=6.00; P=0.02**	**$F_{1,132}$=17.51; $P=5.18\times10^{-5}$**	$F_{1,132}$=0.16; P=0.69
Experiment 2					
Individual body mass (average of 50 individuals per replicate, µg)	Linear model	20	$F_{1,16}$=1.98; P=0.63	$F_{1,16}$=11.32; P=0.25	$F_{1,16}$=0.01; P=0.93
Multivariate metabolic profile (37 molecules)	RRPP – permutational MANOVA	20	**$F_{1,16}$=2.10, P=0.03**	**$F_{1,16}$=2.33, P=0.02**	$F_{1,16}$=1.68, P=0.06

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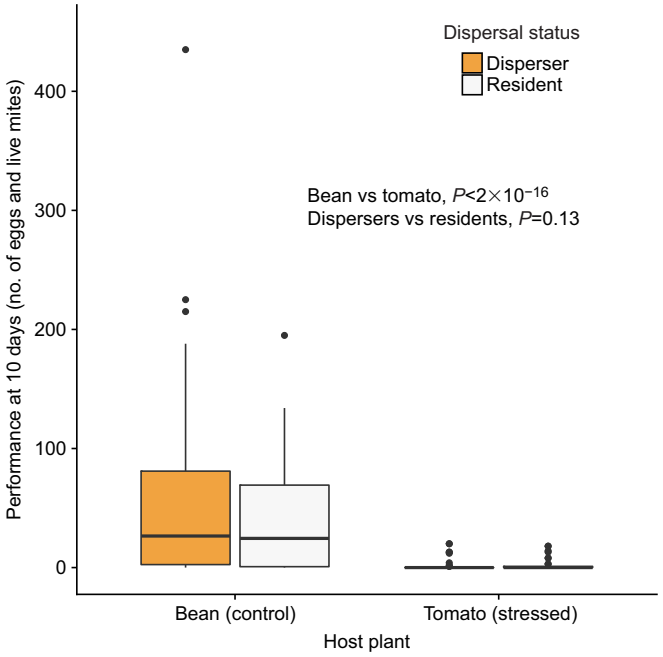

Fig. 2. Effect of dispersal status and host plant on mite performance, measured as the number of live individuals (all stages, including eggs) present on leaves after 10 days in experiment 1. See Table 1 for test details. Sample sizes: 40 independent mites per dispersal×host combination.

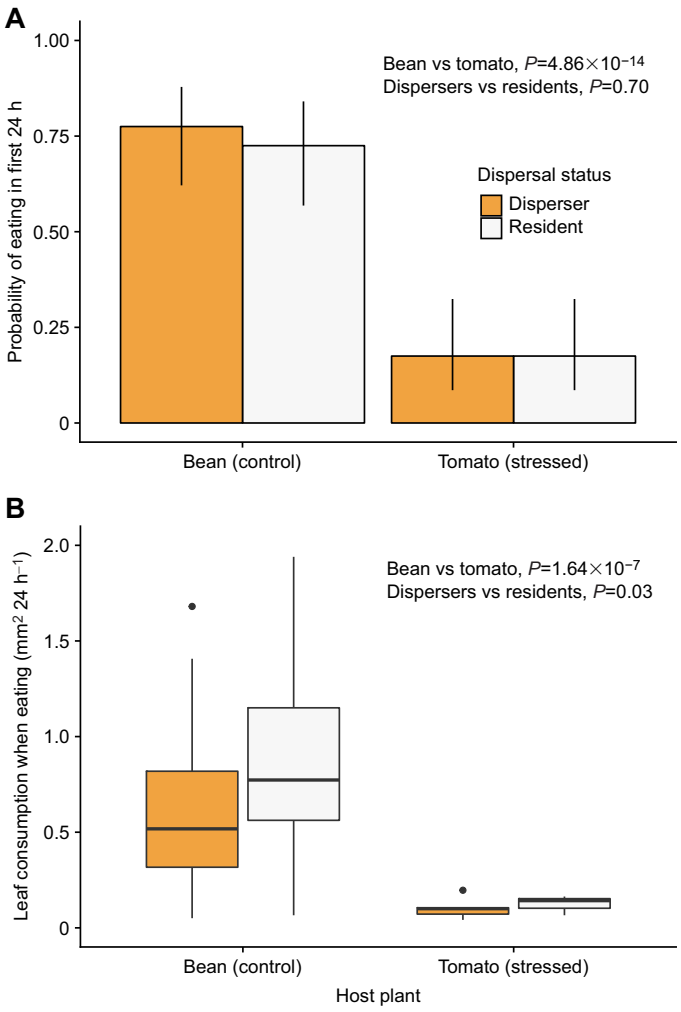

Fig. 3. Effect of dispersal status and host plant on spider mite feeding. (A) Feeding probability per 24 h with 95% CI; (B) feeding intensity (leaf damage per 24 h) in the 74 mites that did feed during the first 24 h of experiment 1. See Table 1 for test details. Sample sizes, from left to right: (A) 40, 40, 40, 40 independent mites, (B) 31, 29, 7, 7 independent mites.

than mites kept on bean (10.27±0.18 versus 9.16±0.19×10^5 µm^3; Fig. 4, Table 1). There was again no significant dispersal×host interaction.

Experiment 2: Effects of dispersal status and host plant quality on changes in metabolic profiles

Mean mite fresh mass was independent from dispersal status, host plant or their interaction (ANOVA, mean±s.e.m. individual mass=12±1 µg; Table 1). The multivariate metabolic profile was significantly influenced by both dispersal status and host treatment, but with no significant interaction among these terms (Table 1). The molecular category-level GLMM explained 52.36% of the total variation in concentrations (R^2_m) with 13.37% explained by fixed effects (inter-category variations and category×treatment effects, R^2_c). The model successfully predicted mean relative concentrations for each treatment×molecular category (Pearson correlation between predicted and observed values=0.95) and for each treatment×molecule (r=0.97) combination (see Figs S3–S8). Metabolite mean relative concentrations were significantly influenced by dispersal status and host plant, but not by their interaction (see Table 2). Analysis of model coefficients indicates that residents have on average higher concentrations of amino acids and amines than dispersers (Table 2, Fig. 5). Tomato-exposed mites, in contrast, have on average lower concentrations of sugars than bean-kept controls (Table 2, Fig. 5).

DISCUSSION

By combining dispersal assays, ‘classical’ phenotypic trait measurements and metabolomics, we developed an unprecedented multi-level study on dispersal syndromes and the way they may be shaped by environmental context (Bonte and Dahirel, 2017). Our experiments show that the spider mite *T. urticae* exhibits a dispersal syndrome, as dispersers and residents differed in several of the traits we studied, including metabolic profiles rarely investigated in this context. However, contrary to our hypotheses and despite clear costs of biotic stress exposure, phenotypic differences between dispersers and residents were not influenced by host plant. This is especially surprising as exposure to a stressful host had, by itself, major consequences for mite phenotype, influencing all tested traits (except adult body size, which was reached prior to exposure; Table 1), including traits involved in the dispersal syndrome. This suggests that maintaining fitness prospects by resisting stress or by escaping unfavorable habitats are unrelated strategies that may be selected independently.

Mites from a bean-adapted lineage performed worse and fed much less when left on tomato compared with bean controls (Figs 2 and 3). Most tomato-exposed mites even stopped feeding altogether (Fig. 3). Additionally, metabolic profiles of tomato-exposed mites were specifically characterized by lower concentrations of sugars (Fig. 5). This is a physiological signature of a starved state, characterized by individuals relying on, and depleting, their body reserves, including carbohydrates (e.g. Dus et al., 2011; Shi et al., 2017; Zhang et al., 2019). Surprisingly, there were no other metabolic differences between tomato- and bean-exposed mites,

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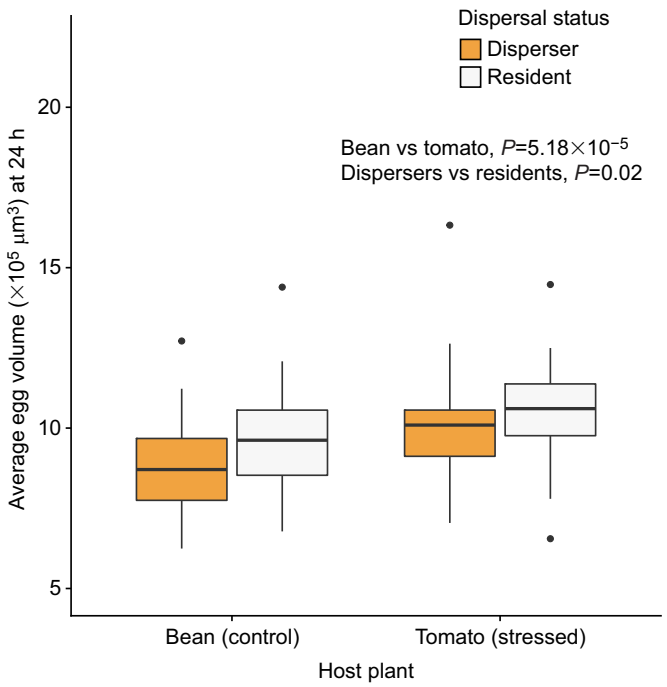

Fig. 4. Mean egg volume per mother as a function of dispersal status and host plant (for the 136 mites that laid eggs in the first 24 h of experiment 1). See Table 1 for test details. Sample sizes, from left to right: 32, 32, 36, 36 independent mothers.

despite amino acids also being a potential source of energy under stressful contexts (Zhang et al., 2019), several organic acids being involved in the energetic metabolism (lactic and citric acid cycles), and accumulation of compatible solutes, in the form of polyols, being documented in response to several stressful contexts, including food stress (such as substrate enriched in urea owing to high population densities; Henry et al., 2018). Nonetheless, our results are in line with those of previous studies. Indeed, although *T. urticae* is overall a generalist species and resistance to a given stressful host evolves readily (within a few generations), tomato is a consistently challenging host for unacclimated or unadapted mites (Alzate et al., 2017; Marinosci et al., 2015; Wybouw et al., 2015), possibly because of its content in toxic secondary metabolites (Wybouw et al., 2015). However, we found evidence that *T. urticae* is able to partly plastically adjust to such stressful conditions. Mites laid fewer eggs on tomato compared with bean, but they laid larger eggs (Fig. 4). In *T. urticae*, egg size has marked and direct consequences for fitness: larger eggs are more likely to survive to maturity, and yield on average larger adults, sex being equal (Macke et al., 2011). In this haplodiploid species, larger eggs are also more likely to be fertilized, and hence female (Macke et al., 2012). Our results align with those of a previous study showing that female *T. urticae* having experienced, or experiencing, poor dietary environments are more likely to lay female eggs (Wrensch and Young, 1983). Female larvae are more likely than males to survive food stress during development (Wrensch and Young, 1983). Laying fewer but larger eggs on tomato may thus be a plastic response increasing mite initial success chances on a challenging host plant, and thus the odds of eventual successful genetic adaptation (Crispo, 2007; West-Eberhard, 2003). Note that, with our experimental design, we unfortunately cannot disentangle these three consequences of host-plant-induced changes in egg size (higher survival, larger size, altered sex ratio) on the second generation. Indeed, we did not raise eggs in isolation (contrary to e.g. Macke et al., 2011), and treatments with larger eggs also had lower population density after egg hatching. Nonetheless, the various consequences of tomato exposure for mite physiology, feeding and life history are all compatible with plastic responses to food stress induced by feeding deterrence, possibly associated with direct toxicity of some tomato secondary metabolites.

We found that dispersers and residents differed in a suite of physiological, life-history and performance traits, providing evidence of a complex multivariate dispersal syndrome in *T. urticae*. Dispersers and residents had different metabolic profiles, mostly owing to the former having, on average, lower concentrations in amino acids than the latter (Fig. 5, Fig. S4). Surprisingly, given the energetic costs thought to be associated with dispersal (Bonte et al., 2012) there were no differences in carbohydrates and organic acids (some of which are involved in the citric and lactic acid cycles) between dispersers and residents (Fig. 5). This may be because dispersers are tested after dispersal, when they may have spent this excess of energetic reserves; however, based on our observations, the expected duration of dispersal should be short (<1 h) compared with the time available to replenish reserves post-dispersal (the rest of the dispersal assay time+24 h of the stress test). Given similar resource quality,

Table 2. Model coefficients for the generalized linear mixed model (gamma family, log link) explaining metabolite relative concentration as a function of dispersal, host plant and metabolite molecular category

	Fixed effect coefficient			
Molecular category	Category intercept	Category×Dispersal status	Category×Host	Category×Dispersal×Host
Amines	−0.20±0.16	**−0.60±0.30**	0.45±0.29	−0.17±0.69
Amino acids	−0.17±0.10	**−0.41±0.18**	−0.30±0.18	0.23±0.40
Carbohydrates (excluding polyols)	**−0.32±0.13**	0.03±0.25	**−0.49±0.24**	−0.44±0.56
Organic acids	−0.07±0.13	−0.05±0.25	0.05±0.24	−0.41±0.56
Polyols	−0.01±0.12	0.18±0.22	−0.02±0.21	−0.43±0.49
Others	−0.20±0.16	−0.37±0.30	−0.55±0.29	−0.56±0.69
Random effect variance				
Molecule intercept	0.05			
Molecule×Dispersal status	0.16			
Molecule×Host plant	0.14			
Molecule×Dispersal status×Host plant	0.99			
Sample intercept	0.08			

Fixed effects are given ±s.e.; bold values are significantly different from zero based on 95% confidence intervals. The model was re-parametrized following Schielzeth (2010) to facilitate interpretation. First, the global intercept was removed and replaced by category-specific intercepts. Second, dispersal and host variables were centered so their coefficients could be interpretable directly even in the presence of interaction (resident or bean=−0.5, disperser or tomato=0.5).

Journal of Experimental Biology (2019) 222, jeb203596. doi:10.1242/jeb.203596

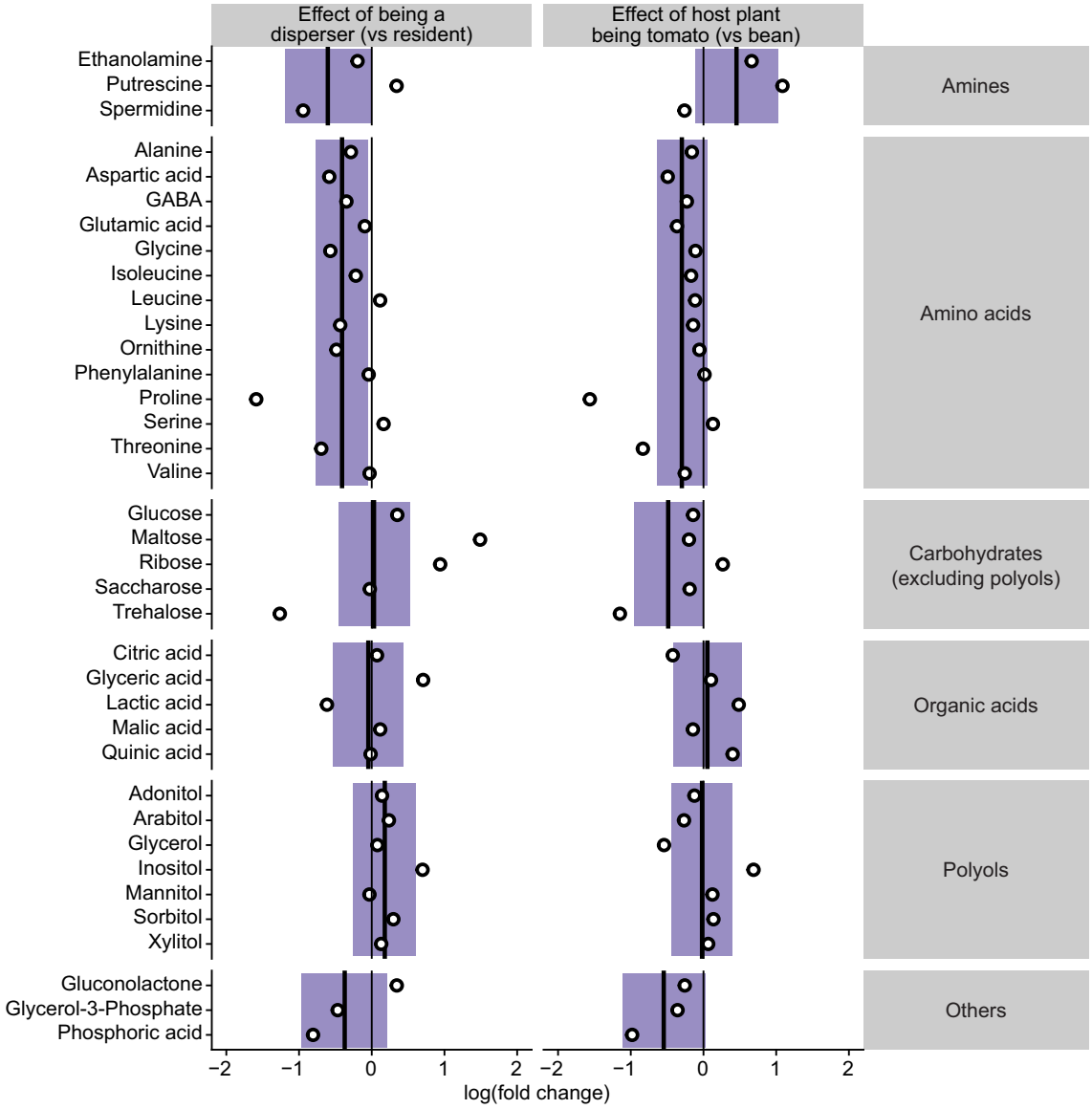

Fig. 5. Effect of dispersal status and host plant on relative metabolite concentration in experiment 2 (Table 2). Molecules are grouped by main biochemical 'categories' based on the Kyoto Encyclopedia of Genes and Genomes (KEGG; Kanehisa et al., 2017); colored rectangles denote 95% confidence bands for the category-level effect of dispersal/host, bold segments the mean predicted effect. Observed mean log(fold change) for each molecule are presented as white-filled dots for illustrative purposes. Sample sizes for dispersers on bean, dispersers on tomato, residents on bean and residents on tomato: 5, 5, 6 and 4 independent groups of 50 mites.

the observed amino acid differences may arise through two non-exclusive mechanisms: differences in amino acid uptake from the host, and/or differences in amino acid production or degradation by mites themselves. Although proof of the latter can only come from deeper physiological studies, our results support at least a partial role of the former mechanism: although dispersers were as likely to feed as residents, dispersers that did feed extracted fewer resources from their host plant per unit of time, based on the lower amount of damage dealt (Fig. 3). Nitrogen/amino acid availability plays a major role in *T. urticae* fecundity and performance (Nachappa et al., 2013; Wermelinger et al., 1985). Accordingly, although dispersers and residents had the same number of offspring (Fig. 2, Fig. S2), dispersers' eggs were notably smaller than residents' (Fig. 4). As mentioned above, egg size has major impacts on offspring future survival and success (Macke et al., 2011; Potter et al., 1976), so adults developed from smaller dispersers' eggs will likely be less competitive against offspring of residents (Bonte et al., 2014). Smaller eggs in dispersers may also result from sex-ratio adjustments by egg-laying females towards more males (Macke et al., 2011, 2012), but we believe it is unlikely in the present case. Indeed, if we assume dispersing females are more likely than residents to find themselves in a newly founded population with few to no other females, then they should produce fewer males, and not more, based on local mate competition theory (Macke et al., 2012).

Altogether, the observed syndrome linking dispersal to feeding, life history and metabolic profile is consistent with the hypothesis of a trade-off between dispersal and foraging efficiency proposed by Fronhofer and Altermatt (2015), to explain population and trait dynamics observed during experimental range expansions. In addition to explaining our present results, the existence of this trade-off in

T. urticae provides a mechanism for the documented fitness costs incurred by dispersers forced to stay in densely populated/resource-limited contexts (Bonte et al., 2014), i.e. in contexts where they would be outcompeted by more 'voracious' residents. By contrast, 'prudent' use of resources by dispersers would be advantageous in newly colonized habitats, where competition with faster eaters is limited (Bonte et al., 2014; Fronhofer and Altermatt, 2015).

However, phenotypic divergence between dispersers and residents was not complete, as they were impossible to differentiate on several tested axes of phenotypic trait variation. There was no difference in body size (length or mass) between dispersers and residents, despite evidence that larger individuals have competitive advantages in mites, including *T. urticae* (Potter et al., 1976; Walzer and Schausberger, 2013), and that body size influences dispersal tendency and/or success in other species (e.g. Moore et al., 2006; O'Sullivan et al., 2014). Although we recorded differences in egg size (see above, Fig. 4), dispersers did not differ from residents in terms of performance at 10 days, hinting at limited differences in egg and larval survival (Fig. 2, see also Fig. S2 for fecundity at 24 h). Our findings in the one-generation experiment are similar to those of Tung et al. (2018) in *Drosophila melanogaster* after several generations of selection for dispersal. They suggested that identical body size and fecundity despite the constitutive and induced costs of dispersal was offset by a shorter lifespan; our experiment was not designed to test this hypothesis. An alternative and non-exclusive explanation for the absence of size or performance syndromes is based on the fact that we only assayed mite life-history traits under low-density conditions in the present study. Indeed, Bonte et al. (2014) showed that dispersers only fared worse than residents in competitive, high-density contexts. We therefore consider it possible that syndromes linking dispersal to body size and fecundity may only be detectable when accounting for plasticity across a range of environmental variation (Bonte and Dahirel, 2017).

Dispersal syndromes were, however, independent from stress exposure, a result that was consistent across life-history traits and metabolic profiling; overall, a mite's dispersal status had no influence on the intensity of its later phenotypic response and tolerance to tomato. Our methodology, which only allowed us to quantify broad categories of common metabolites, may have missed some physiological connections between stress and dispersal syndromes. Indeed, in *D. melanogaster* (Tung et al., 2018), some of the physiological differences between dispersers and residents were due to molecules our own assay could not detect, and that are known to vary in response to some stress (e.g. ATP; Colinet, 2011). However, the fact that this absence of a link is also found in non-physiological metrics makes it less likely to be an artifact of our limited metabolic reference library. This independence of stress response and dispersal was contrary to our expectations based on hypothesized dispersal/stress tolerance trade-offs and contrary to the results of previous meta-population level evolutionary studies (De Roissart et al., 2015, 2016), which showed that spatial contexts selecting for decreased dispersal also selected for higher stress tolerance. Our own results show that this correlated response does not result from pre-existing correlations between these two strategies to mitigate the effects of unfavorable conditions. Dispersal and stress responses are actually independent down to their physiological aspects, which involve shifts in different categories of metabolites (sugars in response to stress versus amino acids in dispersers; Fig. 5). In that context, the apparent population-level correlation between the two traits in evolutionary experiments may result from independent responses to the same selective constraint (Ronce and Clobert, 2012), rather than from pre-existing physiological trade-offs or genetic constraints. For instance, demographic and environmental conditions experienced before dispersal and during development may simultaneously shape dispersal and stress tolerance (Baines and McCauley, 2018; Endriss et al., 2019; Henry et al., 2018; Kent et al., 2009). In particular, individuals that experience stressful conditions prior or during the dispersal window, instead of after in our experiment, may change their dispersal strategy (Mestre and Bonte, 2012) and present other physiological changes (Henry et al., 2018) that may then correlate with dispersal. In *D. melanogaster* for instance, the correlation between physiological variation and variation in foraging behavior (which is now known to be linked to dispersal; Edelsparre et al., 2014) depends on the levels of food stress experienced the day before behavioral assays (Kent et al., 2009).

In any case, this discrepancy between population-level trait shifts after experimental evolution (De Roissart et al., 2015, 2016) and individual-level phenotypic correlations (present study) indicates that dispersal syndromes are not necessarily fixed and may evolve rapidly within species, and that this flexibility needs to be accounted for to fully understand spatial and population dynamics (Bonte and Dahirel, 2017). Experimental evolution approaches involving genetically diverse populations such as the one we used here (Van Petegem et al., 2018) and/or comparative analysis of genetically different lines/populations (Van Petegem et al., 2016) may yield further insights. Complicating the picture is the fact that, in spatially heterogeneous contexts, where dispersal is relevant, individuals may experience variation in not one, but several, biotic and abiotic stressors simultaneously or in close succession (Fronhofer et al., 2018). Because of this, and as more research show that the ecological impacts of stressors can be non-additive (Côté et al., 2016), future studies on the physiological underpinnings of dispersal–stress syndromes should aim to study ecologically relevant combinations of stressors. Such research would fundamentally increase our ability to understand the consequences of stress for movement and forecast its implications for the meta-population dynamics of threatened, range-shifting or pest species in a changing world.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: M.D., S.M., D.B.; Methodology: M.D., S.M., D.R., D.B.; Software: M.D., S.M.; Validation: M.D.; Formal analysis: M.D.; Investigation: M.D., S.M., D.R.; Resources: M.D., S.M., D.R., D.B.; Data curation: M.D.; Writing - original draft: M.D.; Writing - review & editing: M.D., S.M., D.R., D.B.; Visualization: M.D.; Supervision: D.B.; Project administration: D.B.; Funding acquisition: D.R., D.B.

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Data availability
Data are available on Figshare: doi:10.6084/m9.figshare.7857260

Supplementary information
Supplementary information available online at
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