

## RESEARCH ARTICLE

# Lack of alignment across yeast-dependent life-history traits may limit *Drosophila melanogaster* dietary specialization

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## Abstract

Heterogeneity in food resources is a major driver of local adaptation and speciation. Dietary specialization typically involves multiple life-history traits and may thus be limited by the extent to which these traits adapt in concert. Here, we use *Drosophila melanogaster*, representing an intermediate state in the generalist-specialist continuum, to explore the scope for dietary specialization. *D. melanogaster* has a close association with yeast, an essential but heterogeneous food resource. We quantify how different *D. melanogaster* strains from around the globe respond to different yeast species, across multiple yeast-dependent life-history traits including feeding, mating, egg-laying, egg development and survival. We find that *D. melanogaster* strains respond to different yeast species in different ways, indicating distinct fly strain–yeast interactions. However, we detect no evidence for trade-offs: fly performance tends to be positively rather than negatively correlated across yeast species. We also find that the responses to different yeast species are not aligned across traits: different life-history traits are maximized on different yeast species. Finally, we confirm that *D. melanogaster* is a resource generalist: it can grow, reproduce and survive on all the yeast species we tested. Together, these findings provide a possible explanation for the limited extent of dietary specialization in *D. melanogaster*.

## KEYWORDS

 dietary heterogeneity, *Drosophila*–yeast interaction, food-dependent life-history traits, food-mediated adaptation

## 1 | INTRODUCTION

Divergent natural selection in heterogeneous environments can drive local adaptation and speciation (Nosil, 2012; Rundle & Nosil, 2005; Schluter, 2009). Heterogeneity in food resources is a major environmental factor shaping local adaptation in insects, with numerous instances of host plant-driven race formation (Dres & Mallet, 2002; Matsubayashi et al., 2010; Wang et al., 2021). A well-known example is the apple maggot fly, *Rhagoletis pomonella*,

which initially fed on hawthorn but later formed a specialized apple race that adapted to sweeter and earlier ripening apples (Feder & Filchak, 1999; Filchak et al., 2000; Hood et al., 2020). Both extrinsic conditions, such as the heterogeneity of food resources, and intrinsic abilities, such as the enzymatic processing of these resources, determine dietary specialization in insects (Wang et al., 2021). Yet, we have limited understanding of how extrinsic and intrinsic conditions interact to promote or hinder dietary specialization across insect lineages. The exploitation of food resources is a multifactorial

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phenotype that involves a range of traits, including digestion, growth and reproduction. When multiple life-history traits depend on the same dietary resource, dietary specialization will be facilitated by the correlated evolution of these traits (Rothwell & Holeski, 2020). Few studies have systematically compared resource-dependent performance for such a range of traits, limiting our understanding of the mechanisms that shape the evolution of dietary specialization. Here, we use *Drosophila melanogaster* to explore the scope for dietary specialization, by testing the performance of different fly strains on different yeast species for multiple life-history traits.

*Drosophila melanogaster* is described as a dietary generalist, feeding on a broad range of fruits, flowers and fungi, and occurring in most parts of the globe (Markow & O'Grady, 2008). However, there are *D. melanogaster* populations that exhibit differentiation for specific food-derived products (Capy et al., 2000). Moreover, in its ancestral range in Africa, *D. melanogaster* is a seasonal specialist on marula fruit, suggesting that *D. melanogaster* may have evolved from a seasonal specialist ancestor (Mansourian et al., 2018). Within the *D. melanogaster* subgroup, there are both dietary generalist species, such as *Drosophila simulans*, and specialist species, such as *Drosophila sechellia* (Markow, 2015). The intermediate state of *D. melanogaster* as an imperfect generalist, between its seasonal specialist ancestor and its highly specialist relatives, makes *D. melanogaster* an informative model organism for studying the early stages of dietary specialization.

Though *D. melanogaster* is attracted to fermenting fruits and therefore called 'fruit fly', this name is misleading as it is actually the yeast, which metabolizes the sugar contained in fruits and other substrates, that attracts the flies and serves as an essential food source (Becher et al., 2012; Billeter & Wolfner, 2018). Yeast alone is sufficient to attract flies and support larval development, while yeast-free fruits cannot maintain larval survival and are only moderately attractive to flies (Becher et al., 2012). Yeast provides proteins and micronutrients including vitamins, fatty acids and sterols, for fly biology (Baumberger, 1919; Becher et al., 2012; Carvalho et al., 2010; Cooke & Sang, 1970; Piper et al., 2014). Yeast is involved in almost all life stages of *Drosophila*: yeast directly stimulates reproduction, elevating courtship activity by males and remating by females (Gorter et al., 2016; Grosjean et al., 2011); the yeast-inoculated substrate is the preferred substrate for females to oviposit (Becher et al., 2012); yeast increases egg production (Terashima & Bownes, 2004) and contains essential nutrients for larval development (Cooke & Sang, 1970).

There are numerous yeast species that *D. melanogaster* may exploit and that could potentially initiate divergent adaptation. To date, around 7000 yeast species have been described (Kurtzman et al., 2015), and *D. melanogaster* has been found to associate with many of them (e.g. *Candida*, *Hanseniaspora*, *Kluyveromyces*, *Metschnikowia*, *Pichia*, *Saccharomyces*, *Yarrowia*; Stefanini, 2018). Many species from these genera are globally distributed but occur not only in different (micro)habitats including fruits, flowers, mushrooms and soil but also in anthropogenic habitats such as wineries and breweries (Kurtzman et al., 2015). Different yeast species vary

in their fermentation capacities, physiological growth profiles on different substrates and the production of metabolites (Kurtzman et al., 2015), thereby potentially differing in their effects on fly biology. In nature, several aspects of yeast may be heterogeneous simultaneously, deriving from the substrates where it grows, the spatial distribution of these substrates and the fermentation profiles of yeast on these different substrates. Yet, yeast-dependent adaptation may be primarily driven by the yeast itself, since fermentation on different substrates and for different times tends to generate similar levels of attraction, oviposition and larval survival in *D. melanogaster* (Becher et al., 2012).

In this study, we focus on the heterogeneity of yeast species and take it as a starting point to understand whether yeast diversity, together with the global distribution of *D. melanogaster*, might promote the divergence of *D. melanogaster* strains. Specifically, we tested the biological interactions between four yeast species and seven *D. melanogaster* strains in laboratory conditions, to measure how different yeast species influence fly performance at multiple life-history stages. As *D. melanogaster* depends on yeast in almost all of its life stages, dietary specialization may require that all yeast-dependent life-history traits will be maximized on the same yeast species. We test the extent of this alignment by measuring the fly strains' performance for multiple life-history traits including feeding, mating, egg-laying, egg development and survival, on each yeast species. We also explore the occurrence of trade-offs, where better performance on one yeast species comes at the expense of performance on another, as observed in many cases of insect dietary specialization (reviewed in Hardy et al., 2020).

## 2 | MATERIALS AND METHODS

### 2.1 | Fly strain and yeast species

We selected seven fly strains and four yeast species, thereby generating a diverse panel of interactions (focusing on diversity rather than ecological relevance). The seven wild-type *D. melanogaster* strains (Tai (A), Beijing (B), Dijon (D), Ithaca (I), Netherlands (N), Tasmania (T) and Zimbabwe (Z)), kindly provided by Andrew Clark (Cornell University) and Jean-François Ferveur (University of Burgundy) were originally sampled from Ivory Coast, Africa; Beijing, China; Dijon, France; Ithaca, NY USA; Netherlands, Europe; Tasmania, Australia; Zimbabwe, Africa, respectively (Grangeteau et al., 2018; Grenier et al., 2015; Marcillac & Ferveur, 2004). The four yeast species (*Candida boidinii* (American Type Culture Collection—ATCC, 32195), *Candida californica* (GenBank MG661810), *Kluyveromyces lactis* (Fungal Biodiversity Centre—CBS 2359), *Saccharomyces cerevisiae*) were kindly provided by Ida van der Klei (University of Groningen) and Paul Becher (Swedish University of Agricultural Sciences). *C. boidinii* and *C. californica* were isolated from water and *D. melanogaster*, respectively, while *K. lactis* and *S. cerevisiae* were isolated from dairy and fermenting products, respectively (Becher et al., 2018; Goodman et al., 1984). All seven *D. melanogaster* strains were maintained under laboratory conditions for more than a

decade (Grenier et al., 2015; Murgier et al., 2019). The wild-type *D. melanogaster* lab strain *Canton-S*, which is widely used in *D. melanogaster* research and has been maintained under laboratory conditions for several decades, was included in all assays to validate our methods and to provide a reference for the scientific community. All *Canton-S* data are available in supplementary information.

## 2.2 | Fly husbandry

All adult flies used in this study were reared on fly food medium (recipe: agar (10 g/L), glucose (30 g/L), sucrose (15 g/L), yeast (35 g/L, Red Star active dry yeast, *S. cerevisiae*), cornmeal (15 g/L), wheat germ (10 g/L), soy flour (10 g/L), molasses (30 g/L), propionic acid (5 ml of 1 M) and tegosept (2 g in 10 ml ethanol)). Fly stocks were established by placing 20 males and 20 females into fly-rearing bottles (polypropylene, 177 ml, filled with 45 ml fly food medium). Active dry *S. cerevisiae* was killed by heating to 95°C for 10 min during food preparation. Adults were transferred into fresh bottles for egg-laying once or twice a week and discarded from the bottles after 2–4 transfers. Flies were raised in an incubator (25°C, 12:12h light-dark cycle (LD 12:12) with lights on at 09:00 (ZT 0)) and assayed in the same light and temperature conditions.

## 2.3 | Yeast culture

All experiments were conducted using live yeast harvested from fresh cultures. Yeast species were kept on a solid YPD medium (yeast extract 1%, peptone 2%, dextrose 2%, agar 2%) between experiments. For the experiments, a single colony was cultured overnight in a 500 ml liquid YPD medium (same composition but without agar) in a 2 L glass flask with shaking (200 rpm) at 30°C. Yeast cells were harvested by centrifugation in 50-ml tubes at 3000 rpm for 3 min. After the supernatant was poured off, the mass pellet of live yeast cells was determined by subtracting the tube mass from the mass of the tube with live yeast cells. Yeast suspensions were prepared by diluting yeast cells in autoclaved sterile tap water to approximately 1000 g/L. Yeast suspensions were stored in batches at 4°C and used within 10 days of production.

## 2.4 | Feeding assay

To quantify the amount of yeast consumed by the flies, a modified version of the feeding assay of Jiang et al. (2018) was used. To keep rearing densities at a low and constant level, 20–30 pairs of parental flies were allowed to lay eggs in one fly-rearing bottle for 1 day. Twelve newly eclosed females and four males were collected using CO<sub>2</sub> anaesthesia and aged in fly-rearing vials (25 mm × 95 mm, filled with 6.5 ml fly food) for 5–8 days to get mated females for the test. Around 20 h before the feeding test, flies were transferred to vials (25 mm × 95 mm) filled with 3 ml 1% agarose for

starvation. These vials were put on ice to anaesthetize the flies and we removed all of the males. To quantify yeast consumption, feeding vials were prepared by filling either 0.5% (w/v) dyed yeast prepared by mixing 2 ml live yeast solution with 18 ml 1% agarose at approximately 36°C and 0.1 g blue dye (eriolglaucine disodium, Sigma 861 146) or nondyed yeast (same composition without dye). To start the feeding trials, 11 female flies were transferred from their home vial into a feeding vial (25 mm × 95 mm, filled with 3 ml 1% agarose) with either 450 μl dyed yeast or nondyed yeast. Within 5 min after transfer into the feeding vials, they were placed in an incubator to start feeding for 60 min. Feeding was stopped by freezing the feeding vials at –20°C for at least 1 h. To quantify yeast consumption, all frozen flies from a feeding vial were poured out onto a piece of weighing paper, counted and transferred into a 2 mL safe-seal microtube using a mouth pipette. Then, 500 μl of PBST was added to the tube and the flies were homogenized using a homogenizer (TissueLyser II; QIAGEN) with a metal bead (3 mm) at 30 Hz for 30 s. The tubes were centrifuged for 30 min at 13000 rpm and 100 μl of supernatant was transferred to a 96-well plate. The plate was vortexed for 10 s and the absorbance of the samples at 630 nm was quantified with a plate reader (Isogen Asys, UVM 340). The absorbance of dyed yeast consumed by each fly was calculated by dividing the absorbance of each vial by the total number of flies and subtracting the absorbance of the supernatant from flies fed on nondyed yeast. The absorbance values were converted to dye mass via a standard curve, generated by measuring the absorbance of different dye dilutions and the dye mass was used to calculate the volume of yeast intake.

## 2.5 | Mating assay

Mating behaviour including virginal mating latency, second mating probability and number of matings in the presence of yeast was quantified as described in Gorter and Billeter (2017). Virgin females and males were collected using CO<sub>2</sub> anaesthesia and aged in same-sex groups of 20 in fly-rearing vials for 7–10 days. One day before the mating test, adults were transferred to fresh fly-rearing vials. Mating arenas were prepared by adding 50 μl yeast suspension onto a 35 mm × 10 mm plastic petri dish with 3 ml minimal synthetic medium (agar 2%, dextrose 2%, nitrogen-base without amino acid 6.7 g/L). The arenas were incubated at 30°C for 24 h. To transfer one virgin and one male into the mating arena, a hole approximately 0.3 cm in diameter was pierced in the side of the mating arena and a small paraffin film plug was prepared to cover the hole of the mating arena. Mating arenas were placed in a stainless-steel experiment box (63(D) × 71(H) × 120(L) cm) with white-light LEDs, red-light LEDs and fans. Webcams in the box were connected to a computer with monitoring software (Security Monitor Pro 5.16; DeskShare); one picture was captured every 2 min. A virgin was transferred into the mating arena first, and then, one male from the same strain was placed into this arena using a mouth pipette.

The monitoring of mating started at ZT 5–7, and after 24 h, monitoring was stopped, and all of the pictures were analysed with image viewing software (FastStone Image Viewer). Starting time of the experiment and mating time were used to calculate the mating frequency and mating latency of each pair of flies on the experimental yeast media.

## 2.6 | Egg-laying assay

We measured the number of eggs mated females laid in 24 h on each yeast and used the egg number as a proxy for female fecundity. Virgin females and males were collected using CO<sub>2</sub> anaesthesia and maintained in same-sex groups of 20–25 in fly-rearing vials for 5–7 days. One day before the egg-laying test, flies were transferred to fresh fly-rearing vials. Egg-laying dishes were prepared by pipetting 3 ml minimal synthetic medium (see mating assay) mixed with 0.8% activated charcoal (Velda active filter carbon) into a 35 × 10 mm plastic petri dish. After the medium solidified, a hole approximately 0.2 cm in diameter was made in the middle of the minimal synthetic medium with a plastic pipette tip, and 5 μl yeast suspension was added to the hole. These egg-laying dishes were maintained in a 30°C incubator for 23 h for yeast growth. Two to 4 hours before the experiment, 10–15 pairs of virgin females and males were transferred into a fly vial (25 mm × 95 mm) for mating. One hour before the egg-laying test, the egg-laying dishes were taken out of the incubator and a hole was pierced in the side of the petri dishes in a fume hood using a soldering iron to transfer females into the dishes. The dishes were kept in the fume hood for 1 h to cool. At ZT 6–7, mated females were transferred singly into egg-laying dishes, which were maintained in the same experimental box as for the mating assays. After 24 h, the females were removed and the presence of larvae was checked to confirm successful fertilization. Egg-laying dishes containing eggs laid by mated females were frozen immediately at –20°C for counting the number of eggs. Data from dishes containing eggs laid by virgin females were not used for data analysis.

## 2.7 | Egg development assay

We assayed the development and survival of eggs from the different fly strains on different yeast species to assess offspring fitness on different yeast species. Parental flies were prepared by transferring 70–140 pairs of 2- to 3-day-old mated females and males into a plexiglass cylindrical cage (diameter: 9 cm, height: 15 cm; upper sides of the cages were covered with mesh cloth to prevent fly escape; the bottom sides were connected to a 90 mm petri dish and sealed with tape), and the cages were maintained in a fly-rearing incubator for 2–3 days. Fewer parental flies (70–100 pairs) were kept in the cage for more fecund fly strains, and vice versa. The flies were provided with fly food supplemented with active dry yeast for 2 days before egg collection to boost fecundity (Terashima & Bownes, 2004). One day before egg collection, egg-rearing dishes were prepared by adding

150 μl yeast suspension onto a 55 mm × 15 mm plastic petri dish filled with 5 ml minimal synthetic medium (see mating assay). The dishes were incubated at 30°C for 23 h for yeast growth and placed at room temperature for 1 h before egg collection. To collect eggs, at ZT 0, the bottom food petri dish of the parental fly cages was replaced by fresh food medium petri dishes to allow females to lay eggs for 3–4 h. The replaced fly food petri dish was used to collect eggs. Ten random eggs were collected using a flat head preparation needle and dispersed into an egg-rearing dish around yeast suspension. Eggs were collected and dispersed into dishes within 2 days. Eighty egg-rearing dishes (3–4 replicates of each yeast-strain combination) were then transferred into a stainless-steel experiment box (same box and conditions used for mating, the dishes were dispersed) for automatic photo-taking with the same webcams and monitoring software used in the mating assay. The rest of the dishes were transferred into a fly-rearing incubator (dishes were stacked) to record the developmental time and survival. After the first white pupa (first stage of metamorphosis) was observed, egg-rearing petri dishes in a stainless-steel experiment box were automatically photographed every 2 h, and dishes in the fly-rearing incubator were checked every 3–4 h during the day to count the number of pupae formed thereafter. The assay was stopped when no pupation event was observed for 3 days. We used the same protocol for counting the number of flies that eclosed thereafter, but we started counting adults from the moment of first adult emergence and stopped when no eclosion events were observed for 3 days. Median egg-to-adult time (the time from egg collection to 50% of the total eclosion events in a vial) and egg-to-adult survival (percentage of 10 eggs that successfully eclosed) were calculated as a measure of egg development. Median egg-to-pupae time and egg-to-pupae survival (calculated in the same way as an adult) data are available in the supplementary information (Figure S1).

## 2.8 | Data analysis

The effects of different yeast species on feeding, mating, egg-laying and offspring development of distinct *D. melanogaster* strains were analysed with linear and generalized linear mixed models using the lmer and glmer function, respectively, in the lme4 package (Bates et al., 2015) in R (v 4.0.3, 2020; R Development Core Team, 2020). Yeast species, fly strains and their interactions were included as fixed effects, and yeast batch (culture bottle they came from), yeast day (number of days passed after yeast production), time and the identity of the container in which flies were exposed to yeast were included as independent random effects when applicable. The date of the experiment was included as a random effect, nested in yeast batch and yeast day as applicable. In the feeding assay, starvation time was included as a fixed effect and, as expected, longer starvation time significantly increased yeast consumption ( $\chi^2 = 23.949$ ,  $df = 1$ ,  $p < 0.001$ ). For median egg-to-adult time, the location where egg dishes were placed (in the experiment box or in the incubator) was included as a fixed effect as this affected the light exposure and thereby possibly development. For egg-to-adult survival,

the egg collection date was included as a fixed effect (more adults eclosed from eggs on the 2nd day of egg collection compared with the 1st day). All data were plotted and included in the analyses. The residuals were plotted to inspect heterogeneity and normal distribution. Log-transformation was used for continuous data that were not normally distributed. Overdispersion was checked for count data (i.e. number of matings and number of eggs) using `gof` function from `aods3` package and negative binomial was used as the error structure for the number of eggs. The significance of the fixed effect parameters was tested with the ANOVA function (`car` package, Fox & Weisberg, 2019) and shown in Table 1. Random effects were tested by the backward elimination method in linear mixed models using the `ranova` function from `lmerTest` package and in generalized linear mixed models using the ANOVA function from `car` package. The random effect structure was determined by Akaike Information Criterion (AIC) comparison and shown in Table S1. Since each fixed effect parameter has multiple levels, we performed multiple comparisons using the post hoc Tukey test (`emmeans` package, Lenth, 2020) to identify the differences between all combinations of fly strains and yeast species. For each fly strain, the standardized mean value of each trait ('performance') on different yeast species was used to rank the yeast from the worst (the lowest standardized mean value) to the best (the highest standardized mean value). A ranking heat map was plotted to visualize the alignment between life-history traits. For developmental time, we used the reciprocal of egg-to-adult time to rank the yeast (i.e. fastest development = best). We obtained the overall fitness value of each fly strain on each yeast by summing each standardized trait value (feeding, remating probability, number of eggs, egg-to-adult time and egg-to-adult survival) of each strain on each yeast species. Hypothetical best and worst performances were calculated by summation of each maximal or minimal standardized trait value for each strain, derived from any of four yeast species, to provide reference values for comparing the suitability of each yeast species for each fly strain.

## 3 | RESULTS

### 3.1 | Yeast consumption

We observed significant variation in yeast consumption between fly strains and between yeast species (Table 1, Figure 1). For all four yeast species, strains A, B, D and Z consumed more yeast than strains I, N and T (Table S2, Figure 1). Some of this variation may be due to differences in body size between strains (for example, individuals of strains B and D tended to be larger than those of strain N; personal observation). Three out of the seven strains showed within-strain differences in consumption between yeast species (Table S3). For example, strain D and I consumed more *C. californica* than *K. lactis* (Table S3, Figure 1). We also found a significant fly-by-yeast interaction (Table 1), indicating that fly strains significantly differed in how much of each yeast species they consumed. For example, strain N

consumed more *S. cerevisiae* than *C. boidinii*, while the other strains consumed similar amounts of these yeast species (Figure 1).

### 3.2 | Mating behaviour

Fly strains differed quantitatively in mating behaviour, with virginal mating latency, second mating probability and the total number of matings in 24 h varying significantly (Table 1, Figure 2a–c). Exposure to different yeast species did not influence virginal mating latency and the number of matings in 24 h (Table 1, Figure 2a,c), but significantly influenced the probability of second mating (Table 1, Figure 2b). For instance, fly strain N remated less on *C. californica* than on the other three yeast species. This effect of yeast on second mating probability is consistent with previous reports that mated females are tuned to yeast more than virgin females (Gorter et al., 2016). We also found that fly strain and yeast species interacted to affect virginal mating latency, but not for the other two measurements of mating (Table 1). For example, virgin females of strain B mated more slowly on yeast *K. lactis* than on *S. cerevisiae*, while virgin females of the other strains had similar mating latencies on these yeast species (Figure 2a).

### 3.3 | Number of eggs laid in 24 h

Fly strains differed in the number of eggs laid in 24 h (Table 1, Figure 3). For example, strain A and D laid more eggs than strain I, N and T (Table S2). Exposure to different yeast species significantly affected the number of eggs laid in 24 h in strain I and Z: strain I laid more eggs on *S. cerevisiae* than on *C. boidinii* and strain Z laid more eggs on *K. lactis* than on *C. californica* (Table S3, Figure 3). Fly strain and yeast species interacted to modulate the number of eggs laid in 24 h (Table 1). For example, strain Z laid more eggs on *K. lactis* than on *C. californica*, while the other strains laid a similar number of eggs on these yeast species.

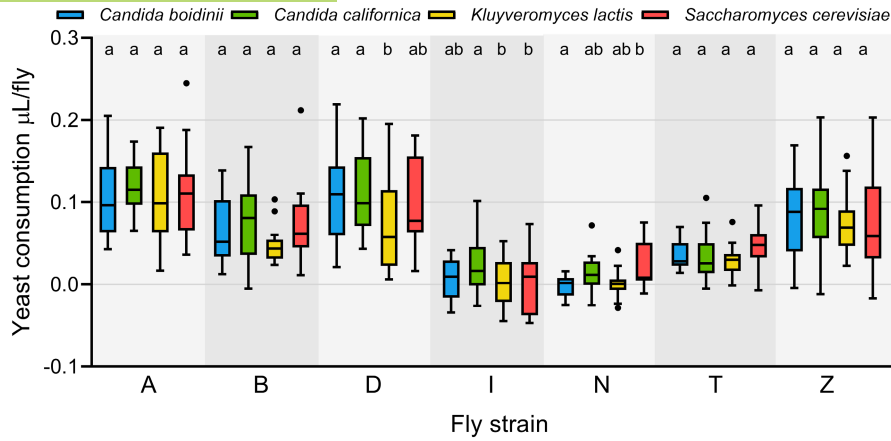
### 3.4 | Egg development

Fly strain, yeast and the interaction between these two factors played a significant role in modulating median egg-to-adult time (Table 1, Figure 4a). For example, strain A, B, N, T, Z eclosed faster on *S. cerevisiae* than on *K. lactis*, while strain D and I eclosed at similar speed on these yeast species. Fly strains significantly differed in egg-to-adult time on yeast *K. lactis* with strains A, B, N, T and Z eclosing later than strains D and I (Table S2). In line with egg-to-adult time, strain and yeast also interacted in modulating egg-to-adult survival (Table 1). For example, a similar number of strain Z adults eclosed on yeast *C. californica* and *C. boidinii*, while more adults of the other strains eclosed on yeast *C. californica* than on *C. boidinii* (Figure 4b). This suggests that not all yeast facilitate fly survival equally well—even if all fly strains accept the yeast for feeding, mating and egg-laying. Yeast species significantly affected egg-to-adult survival for all strains except strain Z (Figure 4b, Table S2).

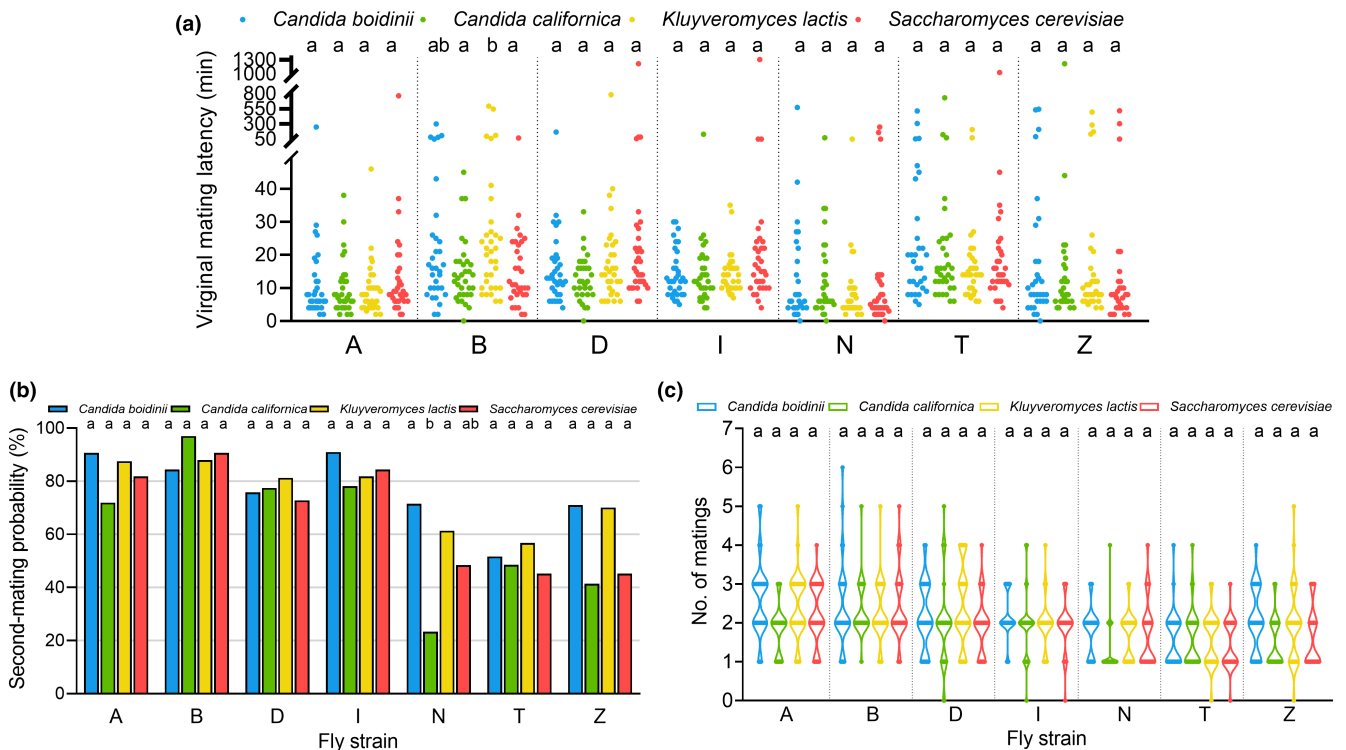
TABLE 1 Analyses of the effects of yeast and fly strain on feeding, mating, egg-laying and egg development

Trait	Metric	Model	n	Fixed effects	$\chi^2$	df	p Value
Feeding	Yeast consumption	lmer(lgYeastCon ~ Yeast*Fly + StarvationTime + (1 YeastDay/Date) + (1 YeastBatch/Date))	507	Yeast	26.272	3	<0.001
				Fly	640.239	6	<0.001
				Yeast:Fly	29.865	18	0.0388
Mating	First-mating latency	lmer(lgML1 ~ Yeast*Fly + (1 YeastBatch/Date) + (1 YeastDay/Date) + (1 DishNumber))	884	Yeast	2.9314	3	0.40232
				Fly	90.5767	6	<0.001
				Yeast:Fly	29.6505	18	0.04099
Second mating probability	Second mating probability	glmer(MP2 ~ Yeast*Fly + (1 YeastBatch/Date) + (1 YeastDay/Date) + (1 DishNumber), family = binomial)	884	Yeast	14.102	3	0.00277
				Fly	83.855	6	<0.001
				Yeast:Fly	18.993	18	0.392
Mating frequency	Mating frequency	glmer(Matings ~ Yeast*Fly + (1 YeastBatch/Date) + (1 YeastDay/Date) + (1 DishNumber), family = poisson)	891	Yeast	7.272	3	0.06371
				Fly	38.225	6	<0.001
				Yeast:Fly	7.016	18	0.9899
Egg-laying	Number of eggs	glmer.nb(Egg ~ Yeast*Fly + (1 DishNumber) + (1 YeastBatch/Date) + (1 YeastDay/Date))	775	Yeast	21.18	3	<0.001
				Fly	281.870	6	<0.001
				Yeast:Fly	28.982	18	0.04861
Egg development	Egg-to-adult time	lmer(Adu50 ~ Yeast*Fly + Location + (1 Time) + (1 Date))	286	Yeast	173.16	3	<0.001
				Fly	44.662	6	<0.001
				Yeast:Fly	90.275	18	<0.001
Egg-to-adult survival	Egg-to-adult survival	glmer(cbind(Adult, Dead) ~ Yeast*Fly + Date + (1 Time) + (1 Location) + (1 DishNumber), family = binomial)	291	Yeast	130.679	3	<0.001
				Fly	56.243	6	<0.001
				Yeast:Fly	91.67	18	<0.001

Note: Significant effects ( $p < 0.05$ ) are shown in bold.



**FIGURE 1** Yeast consumption of fly strains. Volume of yeast consumed by individual mated females in a 1-h period. Boxes present medians (horizontal lines) with interquartile ranges. Outliers are represented by black symbols. Number of replicates for each yeast-fly combination ranges from 15 to 22. Note that yeast consumption estimates can be negative when flies consume very little yeast or excrete it early after the start of trials. Significant differences between yeast species for each fly strain are indicated with different letters. For all comparisons between yeast-fly combinations, refer Table S4



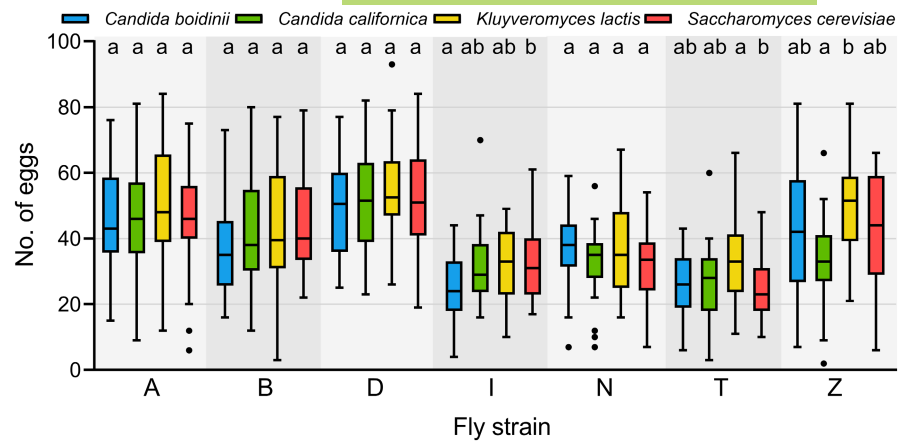
**FIGURE 2** Mating behaviour of fly strains on different yeast species for single male–female pairs in a 24-h period. (a) Virginal mating latency. Number of replicates ranges from 27 to 33. (b) Probability of second mating. Number of replicates ranges from 28 to 33. (c) Number of matings in a 24-h period for single male- single female pairs. Horizontal bars are the result of overlapping symbols. Number of replicates ranges from 28 to 33. Significant differences between yeast species for each fly strain are indicated with different letters. For all comparisons between yeast-fly combinations, refer Table S4

### 3.5 | The effects of yeast on fly strains are not aligned across life-history traits

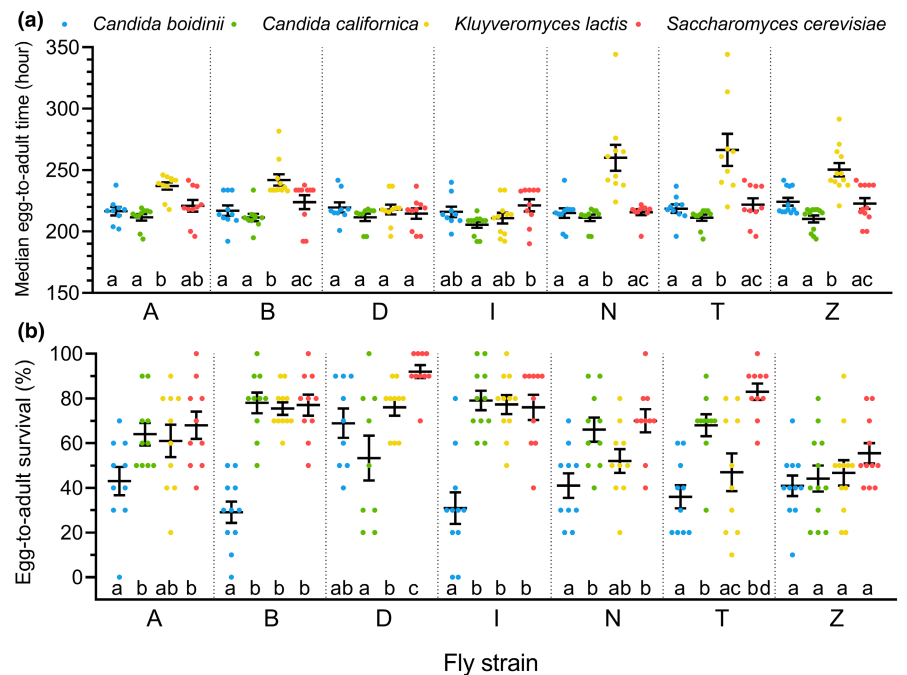
We measured the alignment between yeast-dependent life-history traits with their performance ranks on different yeast species. This shows that different traits are maximized on different yeast species

and none of the yeast species is consistently favourable or unfavourable for any given fly strain (Figure 5a). This is also seen when we plot the cumulative fly performance levels in relation to the hypothetical lowest and highest fitness levels for each fly-yeast combination: the observed values all lie in-between the hypothetical worst and best (Figure 5b). This confirms that none of the yeast species

**FIGURE 3** Egg-laying of fly strains on different yeast species. Number of eggs one mated female laid in 24 h. Boxes present medians (horizontal line) with interquartile ranges. Outliers are represented by black symbols. The number of replicates for each yeast-strain combination ranges from 18 to 31. Significant differences between yeast species for each fly strain are indicated with different letters. For all comparisons between yeast-fly combinations, refer Table S4



**FIGURE 4** Egg-to-adult development of fly strains on different yeast species. (a) Median egg-to-adult time. (b) Percentage of egg-to-adult survival. Symbols represent the mean and standard errors of the mean. The number of replicates for each yeast-strain combination ranges from 9 to 12. Significant differences between yeast species for each fly strain are indicated with different letters. For all comparisons between yeast-fly combinations, refer Table S4



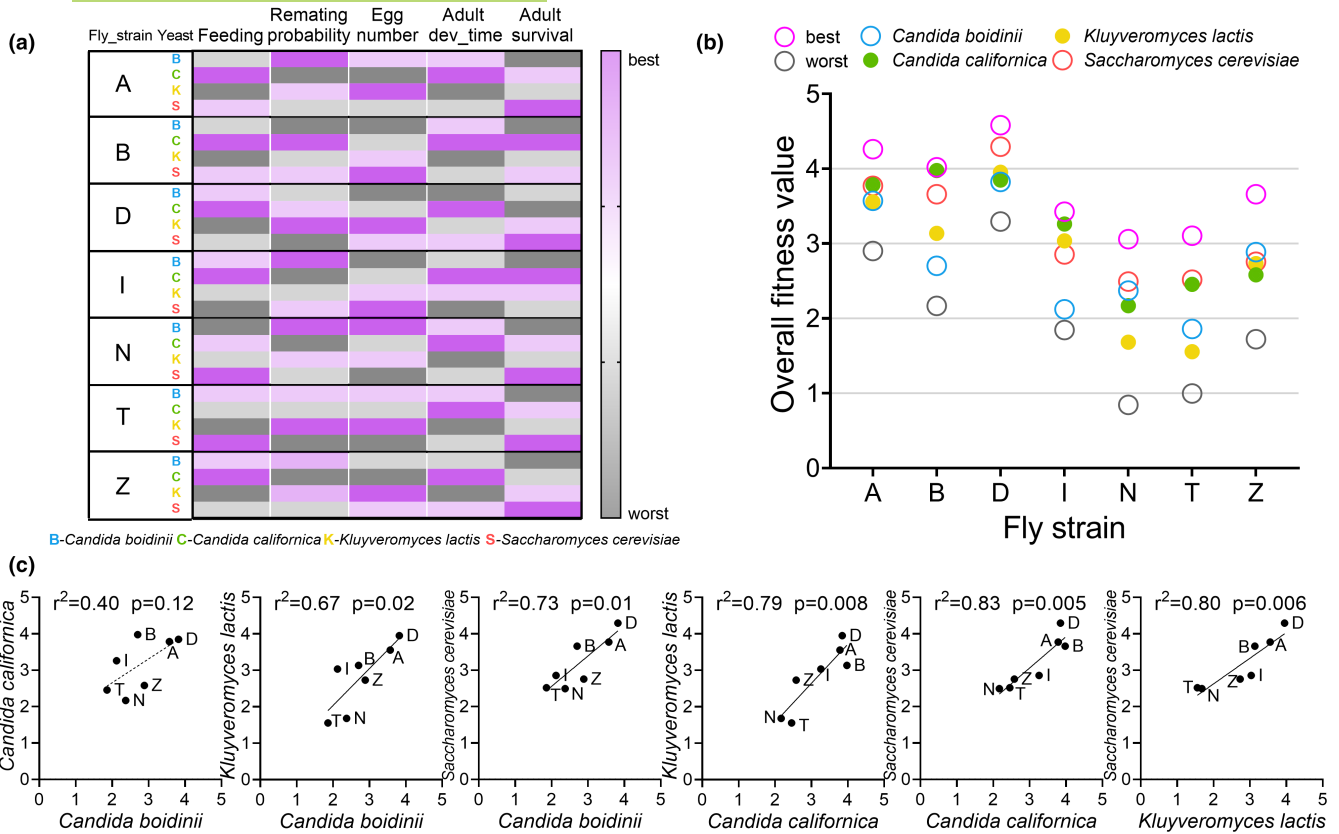
consistently supports or hampers the performance of flies across all yeast-dependent life-history traits. Moreover, the yeast species that maximizes (or minimizes) fly performance differs between fly strains, indicating the potential of yeast-specific differentiation. We also observe that trade-offs in performance on different yeast species are absent: performance on alternative yeast species is positively correlated (Figure 5c). However, different fly strains differ in the extent to which they may be subject to trade-offs (Figure S3). For example, strains B, I, N and T are more likely to be subject to trade-offs in performance on alternative yeast species compared with strains A, D and Z since they show larger variation in performance on alternative yeast species (Figure S3).

## 4 | DISCUSSION

In this study, we explored whether yeast diversity can promote divergence between *D. melanogaster* strains, by measuring a series of

yeast-dependent life-history traits for multiple fly strains and yeast species. We have three main findings. First, fly strains vary in their responses to different yeast species: some strains perform well on a specific yeast species, while other strains do not. Second, trade-offs in performance on different yeast species are absent, but fly strains differ in the extent to which they may be subject to such trade-offs: some strains exhibit larger variation in performance on alternative yeast species than others. Third, yeast-dependent trait responses are not aligned: different life-history traits are maximized on different yeast species. In addition, our results confirm the existing insight that *D. melanogaster* is a resource generalist: it can grow, reproduce and survive on all the yeast species we tested. Taken together, our findings suggest that yeast species diversity could in principle initiate food-mediated differentiation among fly strains, but such differentiation may be hampered by the absence of strong trade-offs in performance between different yeast species, and the lack of alignment among yeast-dependent life-history traits on the same yeast species.





**FIGURE 5** Lack of alignment between yeast-dependent life-history traits on different yeast species for different fly strains. (a) Ranking heat map for different yeast-dependent life-history traits (feeding, remating probability, number of eggs, egg-to-adult time and egg-to-adult survival) of fly strains on different yeast species. Yeast species were ranked from worst (dark grey) to best (purple) for fly performance. (b) Standardized overall fitness value of fly strains on different yeast species and hypothetical best and worst performance of each fly strain. (c) Correlation of fly performance on alternative yeast species using standardized overall fitness values of fly strains on different yeast species

We find that *D. melanogaster* can use yeast species they might rarely encounter in their natural environment, such as *C. boidinii* and *K. lactis* that we used in our study, indicating that *D. melanogaster* can utilize novel diets. However, the yeast species we presented have different effects on performance. For instance, *C. boidinii* generates very low egg-to-adult survival, while *S. cerevisiae* in general generates high egg-to-adult survival. We speculate that the low survival in *C. boidinii* is because *C. boidinii* blocks larvae from breathing fresh air since we observed that *C. boidinii* tended to overgrow the surface of the medium. However, further studies are needed to elucidate these and other mechanisms underlying differences in fly performance across yeast species. We show here that fly strains from different geographic origins vary in how they perform on different yeast species, indicating that these strains are genetically distinct in interacting with yeast. This strain variation indicates some opportunities for specialization on specific yeast species by *D. melanogaster*. Notably, for some traits like egg-to-adult development time and survival, we observed very strong interactions (Table 1). For other traits including feeding, virginal mating latency and number of eggs, we only found marginally significant interactions (Table 1). Yet, even in these cases, we detected highly significant differences in specific fly-yeast combinations. For instance, strain I consumed more *C. californica*

than *S. cerevisiae*, while strain N consumed more *S. cerevisiae* than *C. californica* ( $\chi^2 = 10.7050$ ,  $df = 1$ ,  $p = 0.0011$ ); virgin females of strain B mated faster on *C. californica* than *K. lactis*, while virgin females of strain N mated at equal speed on *C. californica* and *K. lactis* ( $\chi^2 = 10.9604$ ,  $df = 1$ ,  $p < 0.001$ ).

We detected no evidence for trade-offs in fly performance, indicating that flies can use a broad array of yeast species, which is in line with other studies that have failed to find such trade-offs (reviewed in Hardy et al., 2020). We do observe that fly strains differ in this respect: some fly strains show larger variation in performance across yeast species than others, suggesting that constraints to dietary specialization may vary between fly strains. For all fly strains, we found inconsistent effects of yeast on different life-history traits. For example, *K. lactis* that generally supported egg-to-adult survival did not facilitate a high developmental rate of eggs (Figure 4) and *C. boidinii* that was generally supportive for mating generated very low egg-to-adult survival. Expanding on earlier observations of the misalignment between egg-laying preference and offspring development in *Drosophila* (Anagnostou et al., 2010; Koerte et al., 2020; Quan & Eisen, 2018), our study shows that the poor alignment between life-history traits holds true for a suite of yeast-dependent traits and across various fly strains. This suggests that there might be multiple evolutionary constraints for these important life-history

traits to adapt to a novel substrate, potentially hampering dietary specialization in *D. melanogaster*.

In addition to dietary yeast present in substrates, microbes in the gut also play a role in food-mediated local adaptation, by mediating the host's ability to exploit food resources (Brucker & Bordenstein, 2012; Shropshire & Bordenstein, 2016). Flies may or may not require (and harbour) microbial communities for efficient exploitation of a novel food resource. In our study, we used fly strains that had all been reared for more than a decade on the same standard diet containing *S. cerevisiae*. They are thus expected to have similar microbiota. Consequently, the differences we observed between the different fly-yeast combinations may be a conservative estimate of the variation that may occur under natural conditions. We consider the potential role of gut microbes in dietary adaptation to be a rewarding avenue for future research.

There are indications that divergent selection in adjacent environments can lead to population differentiation in *D. melanogaster* (Capy et al., 2000; Kang et al., 2019; Nevo et al., 1998; Voudibio et al., 1989). In the extensively studied 'Evolution Canyon' in Israel, differences in temperature and humidity between the opposite slopes of the canyon exert strong selection on *D. melanogaster* and have caused adaptive changes in oviposition temperature preferences, viability and longevity (Nevo et al., 1998). Consistent with local adaptation, fly lines derived from the warmer and drier south slope prefer warmer temperatures for oviposition, survive better in warm conditions and better resist desiccation compared with flies from the opposite slope (Nevo et al., 1998). A similar situation was observed in *D. melanogaster* populations in Brazzaville, Congo. An urban population collected from a brewery in Brazzaville was about twice more tolerant to ethanol, harbouring a much higher frequency of the *alcohol dehydrogenase-F* allele than the countryside population, and preferred to mate with urban individuals (Capy et al., 2000). Subsequent studies establish that the two populations are also differentiated in morphological traits including wing and thorax length, thoracic pigmentation and female abdomen pigmentation (Haerty et al., 2003). Both cases imply that ecological contrasts can promote divergence in *D. melanogaster*. However, these seem to represent exceptions rather than the rule: *D. melanogaster* maintains a generalist strategy throughout most of its global distribution. Our study provides a possible explanation for the general lack of diet-induced differentiation in *D. melanogaster*, by showing the flies' capacity to utilize a range of yeast species and the lack of alignment in yeast-dependent performance across traits.

We saw no evidence that laboratory strains of *D. melanogaster* are adapted to *S. cerevisiae*. Although each of the fly strains used in our study was bred on *S. cerevisiae* for more than a decade (i.e. 300–360 generations) and they generally did well on *S. cerevisiae*, they did not display better performance on it than on other yeast species. The same holds true for the laboratory strain Canton-S, which has experienced around half a century of lab breeding on *S. cerevisiae* (Figure S2). These results corroborate other work showing that *S. cerevisiae* is neither preferred over other yeast species nor is the best

for most traits, even though it is most often used in laboratory fly food (Murgier et al., 2019). In the wild, *Drosophila* is rarely associated with *S. cerevisiae* (Buser et al., 2014; Christiaens et al., 2014). Even in wineries and vineyards where *S. cerevisiae* is used for fermentation and *D. melanogaster* and *D. simulans* are commonly found, no *S. cerevisiae* was isolated from fly body surfaces or fly defecation in either species (Lam & Howell, 2015), suggesting that they mainly use other yeast species. Notably, there is substantial genetic variation between *S. cerevisiae* strains in attractiveness to *D. melanogaster* (Christiaens et al., 2014), indicating that the comparison between *S. cerevisiae* and other yeasts should take the strain variation of *S. cerevisiae* into consideration. Moreover, in research laboratories, *S. cerevisiae* used in fly food is typically inactivated or killed at high temperatures during food preparation, thereby losing its fermenting capacity and mainly functioning as a protein source (Grangeteau et al., 2018). Consequently, *S. cerevisiae* used in the fly food may exert selection on egg-to-adult survival during laboratory rearing, but not on other traits such as mating and egg-laying, which are strongly influenced by yeast fermentation products (Gorter et al., 2016; Joseph et al., 2009). For egg-to-adult survival, *S. cerevisiae* maximized performance for five out of seven fly strains (Figure 5a). For other nonselected traits like mating and egg-laying, *S. cerevisiae* maximized performance in fewer strains. Hence, we may conclude that for studying the ecological interaction between yeast and *Drosophila*, researchers should employ live yeast and analyse multiple life-history traits.

To conclude, our study shows that different fly strains perform differently on different yeast species and that the performances of yeast-dependent life-history traits on specific yeast are not aligned. These findings suggest that there are evolutionary constraints for these important life-history traits to adapt in concert, possibly providing a mechanistic explanation of the limited extent of dietary specialization in *D. melanogaster* strains across the globe.

#### AUTHOR CONTRIBUTIONS

JCB, MEM and XCW designed and interpreted the study. XCW performed the experiments. XCW performed the data analysis with feedback from MEM and JCB. XCW wrote the manuscript with contributions of JCB and MEM. All authors approved the contents of this manuscript.

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#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

#### DATA AVAILABILITY STATEMENT

All raw data are available at Dataverse-NL (<https://doi.org/10.34894/T7U6P2>).

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