Heritable intraspecific variation among prey in size and movement interact to shape predation risk and potential natural selection

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KEC, KLM, and JPD designed the study with input of the other authors, all authors performed the study, KEC performed the statistical analyses and led the writing of the manuscript, all authors helped to write the manuscript or contributed to revisions.

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Data Availability Statement

On acceptance, all data and code for the analysis of the data will be permanently archived as a Github repository on Zenodo.

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Abstract

1. Species' traits affect consumer-resource interactions with consequences for population dynamics, food webs, and energy flows through ecosystems. Yet, our expectations for how the strengths of consumer-resource interactions scale with species' traits largely come from interspecific comparisons. It remains unclear whether these interspecific associations hold for intraspecific trait variation among individuals that is subject to natural selection. Few studies have evaluated the heritability of prey traits related to predation risk and their potential genetic correlations to evaluate how selection might operate on intraspecific variation underlying predator-prey interactions.
2. We used outcrossed and then clonally propagated lines of the ciliate *Paramecium caudatum* to examine variation in morphology and movement behavior, the extent to which this variation was heritable, and how intraspecific differences among lines altered *Paramecium* susceptibility to predation by the copepod *Macrocyclops albidus.*
3. We found that the *Paramecium* lines exhibited heritable variation in body size and movement traits. In contrast to interspecific allometric relationships, body size and movement speed did not covary among clonal lines. The proportion of *Paramecium* consumed by copepods was positively associated with *Paramecium* body size and velocity as one might expect from interspecific relationships. However, we also found evidence of an interaction such that greater velocities led to greater predation risk for large body-sized paramecia but did not alter predation risk for smaller body-sized paramecia. The proportion of paramecia consumed was not related to copepod body size. These patterns of predation risk and heritable trait variation in paramecia suggest that copepod predation may act as a selective force that could operate independently on movement speed and body size with the strongest selection against large, high-velocity paramecia.
4. Overall, our results indicate that conclusions drawn from relationships among species in traits and the strengths of consumer-resource interactions need not hold within species. Furthermore, patterns of genetic variation and covariation in traits coupled with their relationships to predation risk can lead to patterns of potential natural selection that would be hard to infer from interspecific patterns alone.

Keywords: Allometry, Body Size, Consumer-Resource, Copepod, Evolution, Foraging, Interaction Strength, Intraspecific Variation, *Paramecium*, Predator-Prey

Introduction

The traits of prey and their predators play an important role in determining the strengths of predator-prey interactions that, in turn, have important consequences for population stability and dynamics, and energy flows through systems (McCann, 2011; Paine, 1980; J. T. Wootton & Emmerson, 2005). Prey and predator traits jointly determine the strengths of predator-prey interactions because each step of the foraging process (e.g. encounters, detections, pursuit) is potentially dependent on predator and prey traits (DeLong, 2021; Jeschke et al., 2002; K. L. Wootton et al., 2023). Because of this dependence on traits, a large body of research exists showing how universal traits such as predator and prey body sizes (and their ratio), the dimension in which interactions occur (2D vs. 3D), the velocities of predators and prey in the environment, and taxonomic identity can determine predator feeding rates (Coblentz et al., 2023; Pawar et al., 2012; Uiterwaal & DeLong, 2020; Vucic-Pestic et al., 2010). For example, several studies have shown that, in general, the parameters that describe predator feeding rates in predator functional response experiments change with body size such that larger predators should have stronger interactions with their prey for a given prey body size (Rall et al., 2012; Uiterwaal & DeLong, 2020; Vucic-Pestic et al., 2010).

The potential for general scaling relationships between predator and prey traits and the strengths of predator-prey interactions is promising (Pawar et al., 2012; Uiterwaal & DeLong, 2020), as interaction strengths can be difficult to quantify and doing so across many interactions can be logistically prohibitive. However, these relationships are generally derived from cross-species comparisons. It is currently unclear whether or in what cases these cross-species patterns are likely to hold for the variation within species. Whereas some studies have found that predator functional response parameters such as the space clearance rate and handling time do change as expected from cross-species relationships with traits like body size within species (Schröder et al., 2016), others have found results that do not or only partially support expectations from cross-species relationships (DiFiore & Stier, 2023; Gallagher et al., 2016; Gibert et al., 2017). For example, whereas cross-species studies generally show an increase in space clearance rates with predator body size, within-species studies and those restricted to particular taxonomic groups often have found a hump-shaped relationship, suggesting that there can be an optimal predator-prey body size ratio that is generally not seen in large scale cross-species comparisons (Brose et al., 2008; Cuthbert et al., 2020; Vucic-Pestic et al., 2010). As intraspecific variation could have important consequences for communities, including through its effects on species interactions (Bolnick et al., 2011; Coblentz et al., 2021; Des Roches et al., 2018), understanding whether or under what circumstances interspecific patterns translate to intraspecific patterns would be valuable.

Despite the potential utility of cross-species comparisons, there are several reasons to suspect that there are cases in which interspecific patterns will not directly translate to intraspecific patterns. First, the scale of variation in cross-species comparisons tends to be much larger than the scale of variation within species. For example, predator body sizes in the FoRAGE (Functional Responses from Around the Globe in all Ecosystems) database range 16 orders of magnitude from flagellates to polar bears (Uiterwaal & DeLong, 2020). Although many species may exhibit large ontogenetic changes in body size, these are often still unlikely to match the range of body sizes in cross-species comparisons. Second, some allometric relationships seen across species may not

scale the same way within species. For example, the canonical '3/4-power scaling law' (aka Kleiber's Law) of metabolic rates with body size often does not hold within species (Glazier, 2005). Third, as mentioned above with intraspecific vs. interspecific patterns of change in space clearance rates with predator and prey body sizes, identical within-species relationships can produce an entirely different pattern at the interspecific level in a manner analogous to Simpson's paradox (a statistical phenomenon in which sub-groups show a different relationship with an explanatory variable than the relationship across all groups with the explanatory variable; (Simpson, 1951)). Overall, then, we may not be able to take for granted that within-species patterns will reflect patterns among species.

In addition to the ecological consequences that intraspecific variation can have, intraspecific variation in heritable traits that are associated with fitness can lead to selection and trait evolution. Despite many studies evaluating how intraspecific trait differences in prey alter predation risk (e.g. Cuthbert et al., 2020; Morgan et al., 2016; Pretorius et al., 2019), it is generally unclear in these studies whether the variation in the traits examined is heritable and to what extent. For many quantitative traits like body size, it is likely that there is at least some component of the variation that is heritable. However, the response to selection will depend on exactly how heritable traits under selection are, and whether genetic correlations exist among traits (Lande & Arnold, 1983). For example, genetic correlations can constrain the possible evolutionary responses or lead to indirect, correlated responses in traits that are genetically correlated with traits that experience direct selection (Arnold, 1992). An integrated understanding of the relationships between intraspecific trait variation, predation risk, and the quantitative genetics of traits can provide a more holistic eco-evolutionary perspective on the likely consequences of intraspecific variation and predation for trait evolution.

Here we take advantage of a laboratory system to examine the extent to which expectations from interspecific relationships describe intraspecific variation, whether that intraspecific variation is heritable, and how it relates to predation risk from a predator. We examined how genetically diverse, outcrossed and then clonally propogated lines of *Paramecium caudatum* varied in morphological and movement related traits, the extent to which those traits are heritable, and how intraspecific variation in *Paramecium* body size and movement traits and body size in the copepod *Macrocyclops albidus* are related to *Paramecium* predation risk. From the results of cross-species studies, we hypothesized that: 1) variation in *Paramecium* body size and movement speed would be heritable and positively correlated, 2) *Paramecium* body size and movement speed would be positively correlated with predation rates, and 3) copepod body size would be positively correlated with predation rates on the paramecia.

Materials and Methods

Study System

We collected our focal species, the ciliate *Paramecium caudatum* from three sites near Lincoln, Nebraska, USA: Spring Creek Prairie Audubon Center (40°41′24″N, 96°51′0″W), Conestoga Lake State Recreation Area (40°45′36″N, 96°51′0″W), and Wildwood Lake State Wildlife Management Area (41°2′24″N, 96°50′24″W) in June and July of 2023. We focused our collections on shallow, nearshore waters with emergent or floating vegetation. When we found paramecia in the water, we isolated individual cells, washed them four times with autoclaved pond water collected from the

Spring Creek Prairie site, and placed the cells alone in separate test tubes. In total, this generated over one hundred isolated lineages. We reared isolated lineages in lettuce media inoculated with bacteria collected from the Spring Creek Prairie site. We made lettuce media using 15g of broken up organic romaine lettuce autoclaved in 1L of filtered pond water with 0.7g of ground dried autoclaved pond mud to supply rare elements. We maintained the bacterial flora in this media by transferring inoculated media into new jars every other day or so.

Conjugation is the sexual stage of paramecia and involves meiosis followed by genetic exchange between individuals of different mating types. To generate outcrossed lines from the isolated lineages of *Paramecium*, in August 2023, we combined cells from all isolates into 100mm Petri dishes to promote conjugation. Cells began conjugating within a day, and we collected adjoined conjugates and isolated them into new tubes. As *Paramecium* exconjugates (the term for individual cells post conjugation) are genetically identical (Ahsan et al., 2022; Bell, 1989; Hiwatashi, 2001), individuals descended from the conjugating pair are clones with the potential to be genetically different from clonal lines that descend from other exconjugant pairs through both recombination and genetic differences among conjugating individuals. We established 132 of these outcrossed and now clonally propagated lines and maintained them on lettuce media.

The focal predator in our foraging experiment, the copepod *Macrocyclops albidus*, was also collected from the Spring Creek Prairie Audubon Center in June through August, 2023. We used a combination of wild-collected adult and lab-reared individuals in foraging trials. For the lab-reared individuals, we isolated gravid *M. albidus* in a single Petri dish with *P. caudatum* provided as food. Eggs hatched and grew through stages, and we collected new adults from these stocks for the trials.

We reared all paramecia and copepod stocks at room temperature (23°C).

Video phenotyping

To examine whether and how the outcrossed *Paramecium* lines differed in morphological and movement traits, we phenotyped cells from videos. Twenty-four hours prior to taking videos of the *Paramecium*, we placed cells from each of outcrossed lines into fresh bacterized media in new test tubes at room temperature to create common-garden conditions. For each of the outcrossed lines, we washed approximately 20 *Paramecium* cells three times in 1mL of 0.2µm filtered autoclaved pond water. We then placed the *Paramecium* onto a Petri dish in 0.1mL of filtered autoclaved pond water and covered the drop with a deep-well projection slide cover (Carolina Deep-Well Slides, Model: 60-3730 60-3730E). Immediately after placing the slide cover over the *Paramecium*, we took a 25s video of the *Paramecium* using a stereo microscope (Leica M165C) outfitted with a camera (Leica DMC 4500). As some of the outcrossed lines did not have enough cells available on the day of video phenotyping, we ended up with videos of 126 of the 133 outcrossed lines.

To extract morphological and movement data from the videos, we used the R package Bemovi (BEhavior and MOrphology from VIdeo; (Pennekamp et al., 2015)). Bemovi uses particle tracking software to identify and track individual cells in videos and then extracts information on the morphology and movement of cells. For each video, we ran the Bemovi analysis and then averaged the extracted data across all the cells within an outcrossed line to get a single set of

average morphological and movement measurements. The number of identified particle tracks used to obtain the averages ranged from N=9-36 identified particles per outrcrossed line after data processing and filtering.

Foraging Experiment

We housed 58 copepods within deep-well projection slides (Carolina Deep-Well Slides, Model: 60-3730 60-3730E) of 2.4cm diameter and 1.4mm depth with approximately 0.8mL of filtered autoclaved pond water. Prior to each foraging trial, we starved the copepods for 24 hours to standardize hunger levels. We used only non-gravid copepods. For copepods that became gravid during the experiment, we fed them paramecia daily until they dropped their eggs and could be used again after a 24-hour starvation period. Before each trial, we washed each copepod twice by removing 0.6mL of water from around the copepod and replacing it with filtered pond water. For each trial, we also washed 40 paramecia three times using 1mL filtered pond water. After adding the 40 washed paramecia to the deep-well projection slide arenas, we placed the slide arenas in an incubator (Percival E30BC8) at 25°C. After 30 minutes of foraging, we removed the slide arenas and counted the number of remaining *Paramecium* cells.

In total, we conducted 4-7 replicate foraging trials with different *M. albidus* individuals for each of 38 randomly chosen clonal lines of *Paramecium*. Across these trials, the same copepod was never used with the same outcrossed line more than once and was never used more than once in a day. Overall, individual copepods were used in 2-8 total foraging trials. This led to a total of 230 foraging trials.

Copepod morphology measurements

We photographed 57 of the 58 *M. albidus* used in the foraging trials using a stereo microscope (Leica M165C) outfitted with a camera (Leica DMC 4500) and measured their lengths and widths in millimeters. The one copepod that was not photographed died during the experiment before photographs were taken, and its foraging trials were removed from the data prior to analysis.

Replication statement for foraging trials

Scale of inference	**Scale at which the factor of interest is applied**	**Number of replicates at the appropriate scale**
Clonal line	Clonal line	4-7
Individual Copepod	Individual Copepod	2-8

Statistical Analyses

Analysis of Phenotype Data

The Bemovi analysis of the videos provided a suite of potential variables to describe the morphological and movement phenotypes of the *Paramecium* outcrossed lines. To reduce the dimensionality of the phenotypes, we first excluded variables that are given by Bemovi but have an unclear meaning in terms of the *Paramecium* phenotypes. These included the mean and standard deviation of the ‘grey-ness’ of the paramecia in the videos and the standard deviations of area, perimeter, length, width, and aspect ratio of the paramecia. Next, we examined a correlation matrix

of the remaining variables averaged across individuals within outcrossed lines to determine which variables were highly correlated with one another and thus may be providing the same or very similar information (see Supplementary Online Material (SOM) 1 for the correlation matrix). For the variables that were highly correlated, we chose one of them to include as a measure of the phenotype. For example, net speed and net displacement were highly correlated and so we chose to only include net displacement as a descriptor of the *Paramecium* phenotype. After this process we were left with the following variables describing the *Paramecium* phenotypes: length, width, aspect ratio (the ratio of length to width), mean turning angle, standard deviation of the turning angle, gross speed, net displacement, standard deviation in the step size, and standard deviation of the gross speed (for definitions of each of these variables see SOM 2). After selecting these variables to describe the *Paramecium* phenotypes, we then further reduced the dimensionality by performing a Principal Components Analysis across the selected variables after centering and standardizing each variable.

We also estimated trait heritability as a measure of the genetic contribution to the variation of each of the selected variables we used to describe the *Paramecium* phenotypes. The amount of measured trait variation among clonal lines can be used to estimate broad-sense trait heritability H^2 (Lynch & Walsh, 1998). To estimate the heritability of traits using clonal lines, one can use an Analysis of Variance (ANOVA) with the trait of interest measured for each cell in the videos as the response and the genotype (or clonal line, in this case) as a fixed effect (Lynch & Walsh, 1998). The broad-sense heritability is then estimated as the amount of variation explained by genotype divided by the total variation (Lynch & Walsh, 1998), with the caveat that maternal effects are not factored out of this estimate of H^2.

Analysis of Copepod Foraging Data

To analyze the copepod foraging data, we used a generalized linear mixed effects model. To allow for over/under-dispersion in the data, we modeled the response (the proportion of paramecia consumed in the foraging trial) as beta-binomially distributed. To account for the lack of independence due to the repeated use of outcrossed lines and individual copepods, we included random intercepts for *Paramecium* outcrossed line and individual copepod. As our questions were about how *Paramecium* phenotypes and copepod size influenced the proportion of paramecia consumed, we included the first and second principal component analysis axes from the analysis of the *Paramecium* phenotypes, their interaction, and copepod length as fixed effects. Copepod length and width were correlated and using width rather than length had no qualitative effect on our results (SOM 3). We performed the regression in a Bayesian framework using the R package 'brms' (Bürkner, 2017). For model details including priors and an assessment of model fit, see Supplemental Material SOM 4. Last, we used predictions from the generalized linear mixed effects model to visualize a 3-dimensional fitness surface in which we defined fitness as the predicted proportion of paramecia surviving after the foraging trial dependent on the first and second principal component analysis axes.

All analyses were performed using R v. 4.3.1 (R Core Team, 2023).

Results

Paramecium Phenotypes

The Principal Components Analysis indicated that the first two Principal Component Analysis axes explained 64.6% of the total variation in the mean phenotypes across all the lines (Figure 1; see Supplemental Material SOM 5 for the full PCA results). The first axis was positively associated with the various measures of speed and the aspect ratio of the paramecia and negatively associated with the mean turning angle of the paramecia. The second axis was positively associated with *Paramecium* size (length, width, and area) and had a slight negative association with the standard deviation in turning angle. Thus, we interpret the first axis as representing movement speed and lack of turning in the paramecia and the second axis as a measure of size. We also note that the fact that size and speed loaded on separate axes reflects that size and speed were not strongly correlated among the outcrossed lines (the absolute values of the correlations between the size and movement traits considered ranged from 0.02-0.36 with a mean of 0.14; SOM 1).

Estimates of the broad-sense heritability of the morphological and movement traits of the paramecia ranged from 0.14 to 0.73 (Table 1). The estimates of heritability of the size traits were 0.64 for length, 0.59 for width, and 0.44 for the aspect ratio. There was greater variation in the estimates of heritability for movement related traits of the paramecia ranging from 0.14 for net displacement to 0.73 for gross speed (Table 1).

Prey and predator traits and foraging rates

We estimated a positive effect of size, speed, and their interaction on the proportion of *Paramecium* cells offered that were eaten by copepods (Figure 2; Table 2). Together these interactive effects were such that small paramecium had relatively constant risk of predation by copepods whereas large paramecium had greater risk of predation when they were fast (Figure 2, Figure 3). We found no statistically clear relationship between copepod size and the proportion of *Paramecium* offered that were eaten by copepods (Table 2).

Discussion

The strengths of interactions between predators and prey have important consequences for the dynamics of populations, communities, and food webs (McCann, 2011; Paine, 1980; J. T. Wootton & Emmerson, 2005). A powerful potential tool for predicting interaction strengths are the relationships between interaction strengths and species' traits (Pawar et al., 2012; Uiterwaal & DeLong, 2020; K. L. Wootton et al., 2023). However, many of the associations between traits and interaction strengths are based on cross-species relationships, and it remains unclear whether these cross-species patterns hold for variation within species. Furthermore, within-species variation can provide an opportunity for natural selection to operate on intraspecific variation in species' traits, but whether and how selection might operate is dependent on whether foraging-related traits are heritable and how traits are genetically correlated (Arnold, 1992; Lande & Arnold, 1983). Using outcrossed and then clonally propagated lines of *Paramecium caudatum*, we examined the structure and heritability of phenotypic variation in size and movement related traits and how these traits were related to predation risk of paramecia by copepods. Overall, cross-species expectations for the relationships between body size and movement and between body size, movement, and predation risk did not match the patterns we observed within *Paramecium*. By examining the structure and heritability of variation in *Paramecium* traits, our analyses also revealed how selection through copepod foraging might operate on *Paramecium* phenotypes.

Studies of allometric scaling have generally shown that species' movement speeds increase with body size (Cloyed et al., 2021; Hirt et al., 2017). Thus, we hypothesized that movement speed and body size would be correlated with one another across our outcrossed lines of *Paramecium*. Our analysis of the *Paramecium* phenotypes, however, showed that variation in speed and body size had largely low correlations. Rather, the morphological trait that was most positively correlated with speed was the aspect ratio of the paramecia. This positive relationship may reflect a hydrodynamic advantage of a larger aspect ratio (Batchelor, 1967), or a greater proportion of cilia being located along the length of the paramecia. Regardless, this independence of size and speed in contradiction of cross-species patterns plays a potentially important role in how paramecia might respond to selection from copepod foraging. Specifically, the independence of size and speed suggests a lack of genetic correlation between the two sets of traits that can potentially allow the paramecia to separately respond to selection on each set of traits (Lande & Arnold, 1983). Coupled with the relationships we saw in which predation risk from copepods was highest on fast and large paramecia, the independence of speed and size suggests that selection due to copepod predation could operate to reduce size, speed, or both with similar fitness results.

Many studies have shown how differences among individuals can lead to differences in interactions with other species including predation risk (e.g. Cuthbert et al., 2020; Morgan et al., 2016; Pretorius et al., 2019). Although many of the quantitative traits considered in these studies are likely to be heritable, this assumption is rarely tested because studies often use ontogenetic differences between individuals, for example, in body size, to examine effects of intraspecific variation or do not take the extra step to determine whether differences have a genetic basis. In our study, there is both ontogenetic variation within outcrossed lines due to differences among individuals in time since cell division and variation due to genetic differences among outcrossed lines. In general, our analyses show that the *Paramecium* traits we considered are heritable despite the ontogenetic variation. We also find that some traits are more heritable than others. For example, all of the size-related traits we examined showed a moderate amount of heritability, whereas the movement-related traits showed greater variation in heritability. Overall, this suggests that certain traits may be able to respond more readily to selection but that selection in the paramecia should be capable of leading to evolution in both size and movement related traits.

Predator and prey relative sizes and velocities in cross-species comparisons show clear relationships with predator feeding rates (Coblentz et al., 2023; Pawar et al., 2012; Uiterwaal & DeLong, 2020; Vucic-Pestic et al., 2010). In general, predator feeding rates increase with increasing predator size and with higher movement velocities in either species (Coblentz et al., 2023; Pawar et al., 2012; Uiterwaal & DeLong, 2020; Vucic-Pestic et al., 2010). Further, the cross-species allometry between size and velocities would suggest higher feeding rates of predators on larger prey due to greater encounter rates (although this could be counteracted by longer handling times in some systems). These patterns led us to hypothesize that: 1) predation risk for the paramecia would increase with *Paramecium* body size and velocity, and 2) predation risk for the paramecia would increase with copepod body size. We found partial support for the hypothesis that *Paramecium* predation risk would increase with body size and velocity. Although we did find positive effects of body size and velocity, we also found evidence of an interaction through which predation risk was highest for large, fast paramecia and was nearly constant for small paramecia regardless of speed. We hypothesize that the reason for this is that smaller paramecia regardless of their encounter rates with copepods may be harder to detect and capture than larger paramecia,

leading to similar predation rates on smaller paramecia regardless of their velocity. However, larger paramecia may be easier to detect and capture, leading to a dependence of predation risk on velocities and encounter rates.

In contrast to *Paramecium* size, we found no evidence of an effect of copepod size on *Paramecium* predation risk. We hypothesize that this may be due to the large body size difference between paramecia and copepods and the relative range of size differences observed in the paramecia versus in the copepods. Mean paramecium lengths among the outcrossed lines used in the experiment ranged from 95-233µm whereas copepod size ranged from 0.9-1.4mm. It is possible that over a larger range of copepod sizes, we would have found an effect of copepod size but that feeding rates on paramecia are generally similar in the size range that occurs among adult copepods of this species. Despite the lack of an effect of copepod body size on *Paramecium* predation risk, our statistical model suggested that there was substantial variation among copepods through the random effect of individual copepod. As the model did not show a statistically clear effect of copepod size and copepod hunger was standardized, these differences may have been due to some uncontrolled factor such as age or behavioral differences among individual copepods (Toscano et al., 2016; Toscano & Griffen, 2014). Nevertheless, together these results show that intraspecific variation in predator and prey traits may lead to effects on predation risk contrary to those predicted from cross-species comparisons.

Conclusions

Species' traits are an important factor determining the strengths of interactions among predators and their prey. Given the large amount of existing data on the relationships between predator and prey traits and the strengths of interactions across species, it would be useful if the patterns emerging from cross-species comparisons would be applicable to the variation in traits occurring within species. Our results suggest that these general, cross-species relationships need not hold for variation within species. Furthermore, by quantifying the heritability and correlations among traits within prey and their relationships to predation, our results also show that expected patterns of potential selection may also be difficult to infer from expectations gathered from cross-species patterns. Overall, we suggest that ecologists and evolutionary biologists use caution when using cross-species comparisons to infer the effects of intraspecific variation and that studies seek to understand the general circumstances under which the effects of within- and between-species variation are likely to be similar or differ.

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Figures

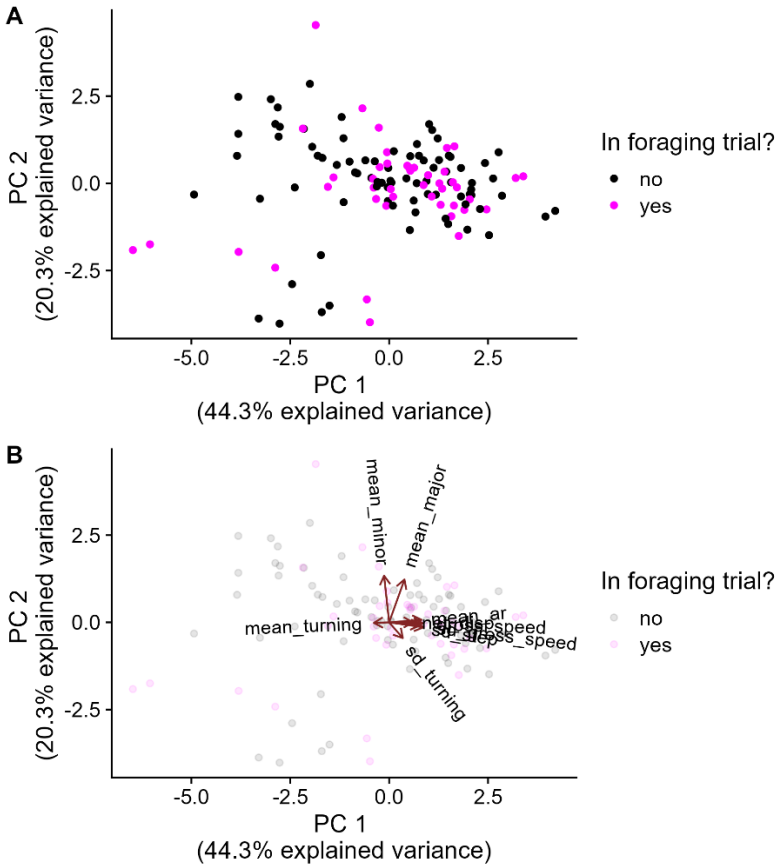

Figure 1. The first two components of a principal components analysis of the mean phenotypes of *Paramecium caudatum* outcrossed lines derived from the analysis of videos explained 64.6% of the total variation (A). The first principal component was positively associated with the mean aspect ratio of the paramecia (mean_ar) and a number of speed related phenotypes (e.g. gross speed and net displacement) and negatively associated with the mean turning angle (mean_turning; B). The second principal component was positively associated with the mean length and width of the paramecia (mean_major, mean_minor; B). For definitions of each of the phenotypic traits included in the principal components analysis see Supplementary Online Material SOM2 and SOM5.

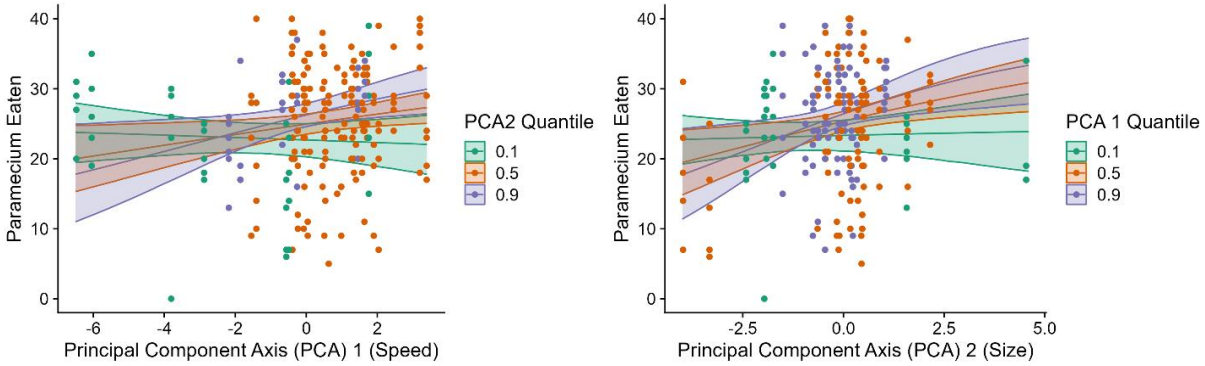

Figure 2. The proportion of paramecium eaten per trial increased with paramecium speed (PCA1) and paramecium size (PCA2). However, there was evidence of an interaction in which the effect of speed was dependent on size such that larger, faster individuals were at greater predation risk whereas small individuals had similar risk regardless of speed. The differently colored lines and shaded areas represent the means and 90% Credible intervals for the relationship between the principal component values and the mean proportion of *Paramecium* consumed by copepods for different quantiles of the principal component not on the x-axis. Points are colored such that the green dots are for outcrossed lines from the 0.15 quantile or less of the principal component not on the x-axis, purple dots are for outcrossed lines from the 0.85 quantile or greater of the principal component not on the x-axis, and the rest of the points are orange.

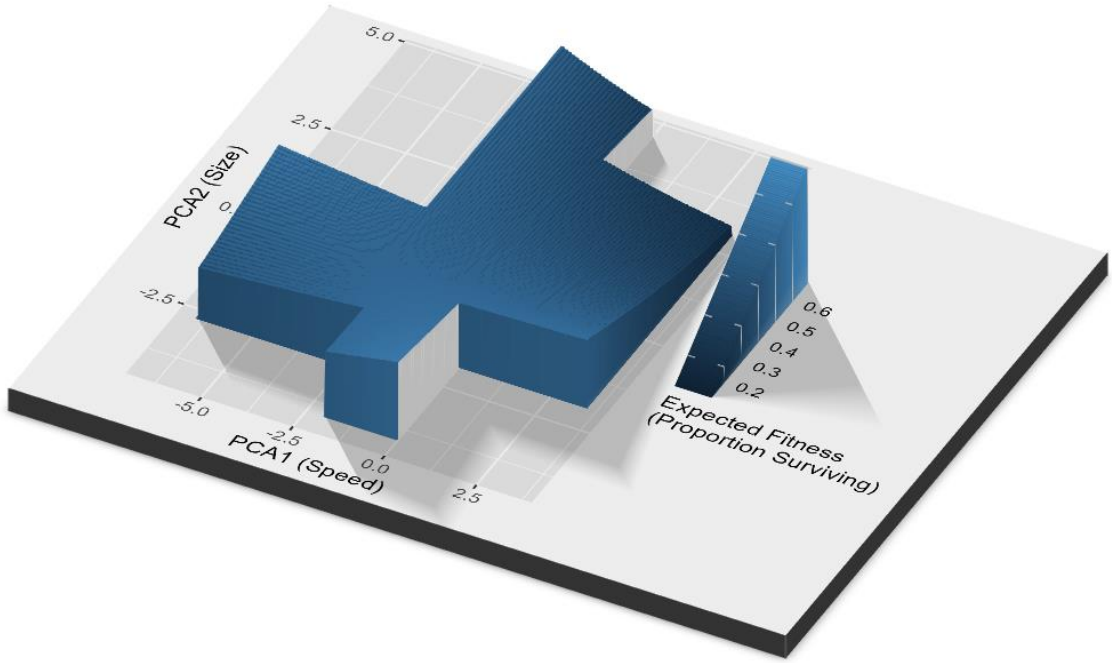

Figure 3. A predicted fitness surface using the expected proportion of paramecia surviving the copepod foraging trials shows that the expected fitness is lowest for paramecia that are both fast (high PCA1 value) and large (a high PCA2 value). Cut out areas of the fitness surface are those in which there were no outcrossed lines with those combinations of PCA1 and PCA2 values.

Tables

Table 1. Summary of quantitative genetics results examining the heritability of *Paramecium caudatum* traits.

Trait	Trait Sum of Squares	Total Variance	Broad-sense Heritability
Major Axis (Length)	1.4×10^6	2.2×10^6	0.64
Minor Axis (Width)	2.0×10^5	3.4×10^5	0.59
Aspect Ratio (Length/Width)	133.3	239.3	0.44
Mean Turning	2.1	13.9	0.15
sd Turning	42.9	111.9	0.38
Gross Speed	3.7×10^8	5.1×10^8	0.73
Net Displacement	2.2×10^9	15.3×10^9	0.14
sd Step Size	2.9×10^6	9.9×10^6	0.29
sd Gross Speed	4×10^7	6.5×10^7	0.62

Table 2. Generalized Linear Mixed Effects Model (GLMM) results examining the relationships between *Paramecium caudatum* traits (PCA 1 and 2) and their interaction and copepod size with the proportion of *P. caudatum* consumed by copepods in feeding trials. Sd in the table stands for 'standard deviation'.

Fixed Effects			
Parameter	**Estimate**	**90% Credible Interval**	**Probability of Direction**
Intercept	-0.09	-2.1,1.8	0.53
PCA 1 (Speed)	0.08	0.01,0.15	0.97
PCA 2 (Size)	0.12	0.03,0.22	0.98
PCA 1 and 2 Interaction	0.05	-0.001,0.11	0.947
Copepod Length	0.51	-1.06,2.2	0.7
Random Effects			
Parameter	**Estimate**	**90% Credible Interval**	
Sd Copepod ID	0.5	0.38,0.65	
Sd Paramecium Line	0.2	0.05,0.37	
Distributional Parameters			
Parameter	**Estimate**	**90% Credible Interval**	
Beta-binomial phi	10.45	8.1,13.6	