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Spatial heterogeneity in resources alters selective dynamics in *Drosophila melanogaster*

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Environmental features can alter the behaviors and phenotypes of organisms, influencing the dynamics of natural and sexual selection. Experimental environmental manipulation, particularly when conducted in experiments where the dynamics of the purging of deleterious alleles are compared, has demonstrated both direct and indirect effects on the strength and direction of selection. However, many of these studies are conducted with fairly simplistic environments, where it is not always clear how or why particular forms of spatial heterogeneity influence behavior or selection. Using *Drosophila melanogaster*, we tested three different spatial environments designed to determine if spatial constraint of critical resources influences the efficiency of natural and sexual selection. We conducted two allele purging experiments to (1) assess effects of these spatial treatments on selective dynamics of six recessive mutations, and (2) determine how these dynamics changed when sexual selection was relaxed and spatial area reduced for two of the mutants. Allele purging dynamics depended on spatial environment, however the patterns of purging rates between the environments differed across distinct deleterious mutations. We also tested two of the mutant alleles, and demonstrate sexual selection increased the purging rate.

KEY WORDS: ***Drosophila melanogaster*, natural selection, selection dynamics, sexual selection, spatial heterogeneity.**

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Understanding mating systems and the dynamics between the sexes can illuminate how sexual selection acts within populations, driving many organisms' behaviors and phenotypes. Key work in the theory of mating systems conducted by Bateman (1948), Trivers (1972), and Emlen and Oring (1977) has led to many studies being dedicated to examining male and female interactions across different species and populations. The mating systems of numerous species have been shown to vary due to local adaption or ecological constraints resulting from environmental factors (Miller and Svensson 2014). For example, ungulate species that inhabit open environments tend towards group mating systems while those within closed or forested environments tend to adopt small group or pair mating systems (Carranza 2000; Bowyer et al. 2020). This variation in behavior can occur within species as well, as seen in the mating system of *Prunella modularis*, which has been shown to shift between polygyny, polygynandry, and polyandry depending on food distribution (Davies and Lundberg 1984). Environmental features such as spatial size, structure, resource abundance, and climate can alter the strength of sexual selection and conflict, leading to fitness payoffs for certain phenotypes. In *Sancassania berlesei*, increasing environmental complexity changes the fitness differences between fighter and scramble male morphs which was believed to be a result of reduced encounters between fighter males (Lukasik et al. 2006). Another example can be found in certain populations of katydid, where sex role reversal occurs under conditions of low resource abundance, placing a greater influence of inter- and intra-sexual selection on females (Gwynne and Simmons 1990). Since environmental variation can impact fitness, it is important to keep environmental context in mind when studying

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the strength of natural selection, sexual selection and sexual conflict.

Along with the environment, understanding the interaction between sexual selection and other components of natural selection (fecundity and viability) is important for determining an organisms' or a populations' phenotypic distribution. Since the term was introduced by Darwin (1871), studies have focused on how traits under strong sexual selection (weaponry, ornaments, and mating behaviors) arise and persist within populations and how this relates to the antagonistic or concordant relationship between natural and sexual selection. For example, when sexual conflict is present, mutations may be beneficial in one sex but deleterious in the other (antagonistic pleiotropy), allowing for the maintenance of conditionally deleterious alleles. In many species, an extreme case of this is males harming females during copulation, either through mating itself or ejaculates, in order to prevent re-mating, further securing the males' paternity (Johnstone and Keller 2000). However, while natural selection and sexual selection are often portrayed as being at odds with one another, individuals of higher overall condition will often on average receive more mates, resulting in sexual selection working in tandem with other components of natural selection. For instance in ungulates, males of overall higher condition tend to have the largest weaponry and are better able to obtain fertilizations along with access to females themselves (Preston et al. 2003; Hoem et al. 2007; Vanpé et al. 2007; Emlen 2008). Martinossi-Allibert et al. (2017) further explored these interactions by examining the strength of selection between male and female seedbeetles under different environmental stressors, observing that environment influences the alignment of sexual selection and other components of natural selection. These examples highlight how interactions between sexual and other components of natural selection influence traits within populations and how seemingly detrimental traits persist within a population.

A common way of determining how various factors influence natural selection is to conduct allele purging experiments. Within these experiments, deleterious mutations are introduced into populations at a known frequency (or via induced mutations) and the rate they are removed from the populations over time is recorded or populations undergo various fitness assays. Experimental conditions are manipulated (thermal stress, dietary stress, population density, environmental complexity, and mate choice; Sharp and Agrawal 2008; Young et al. 2009; Hollis et al. 2009; MacLellan et al. 2009; Wang et al. 2009; Laffafian et al. 2010; Hollis and Houle 2011; McGuigan et al. 2011; Arbuthnott and Rundle 2012; Clark et al. 2012; Maclellan et al. 2012; Singh et al. 2017; Colpitts et al. 2017) and purging rates (or fitness) are compared to obtain estimates of the effects these conditions have on selective dynamics. While several kinds of these studies have been conducted, many show contrasting results in reference to whether sexual selection aids natural selection in the removal of deleterious alleles. One potential reason for such inconsistencies is that most experiments using *Drosophila* are performed in small, simple environments (i.e., small vials) at relatively high densities, and it is not clear the degree to which this may influence the strength and orientation of selection. Such simple and high-density environments likely constrain individuals in terms of mating strategies available in more natural conditions. Alternative mating strategies are density-dependent in several species (Greenfield and Shelly 1985; Höglund and Robertson 1988; Kokko and Rankin 2006), and particularly for *Drosophila melanogaster*, territorial defense strategies by males are less likely to occur when the population is at a high density (Hoffmann and Cacoyianni 1990). Simple environments may also influence female strategies in that they may accept more mates due to being unable to seek refuge or escape from constant male harassment (Byrne et al. 2008). Creating a more "complex" environment consisting of a larger space, multiple food cups, and additional spatial structure to alter the interactions between the sexes, Yun et al. (2017) showed that female harassment of high quality *D. melanogaster* females was greater in the simple vial environments used in many experiments, exaggerating the effects of sexual selection to reduce variance in female fitness.

Since Yun et al.'s (2017) experiment, there have been several studies conducted to determine how natural and sexual selection changes within simple (high density in single vials or bottles) versus "complex environments" (lower density cages with multiple resource patches for interactions to occur). In a later study, Yun et al. (2018) found flies that had mating opportunities within "complex" environments adapted more quickly to novel larval environments as opposed to those mating in simple environments or lacking mate competition. Using a similar environmental design but creating a larger, lower density simple environment, Colpitts et al. (2017) demonstrated that "complex" environments aided the purging of two deleterious mutations that had previously been found to have no difference in purging rate while manipulating opportunity for mate choice (Arbuthnott and Rundle 2012). Singh et al. (2017) showed increased purging rate of deleterious alleles from populations evolving within these "complex" environments, while MacPherson et al. (2018) revealed that low quality females experienced a greater reduction in fitness due to male harm compared to high quality females but only in "complex" relative to simple environments. These studies exemplify that with even modest changes in spatial environment (increasing space and lowering density of individuals), the dynamics of natural and sexual selection can vary vastly. Complexity without the manipulation of overall environment size has been shown to influence female fitness in terms of offspring production (Malek and Long 2019), but this has not been used to test overall population fitness. While these studies potentially show

how these forces interact in a way that may be more representative of what is seen in nature, the types of environments employed are still simple and largely reflect changes in density. However, it is important for such experiments to explicitly consider factors that are known to influence mating strategy as well, such as territory availability and spatial heterogeneity of resources.

Increasing the environmental complexity in which populations evolve may reveal new patterns of how sexual selection acts, particularly for *D. melanogaster*, which as a species shows considerable variability in mating strategy in different spatial contexts. "Typically" displaying scramble competition in the lab, territorial behaviors and resource defense polygyny have been observed when *D. melanogaster* males are given a desirable resource (Hoffmann 1987). Males also appear to display this behavior more often when females are present, when there is a low density of males, and the resource is readily used by females for oviposition and resource patches are in a range of sizes (∼20mm diameter) (Hoffmann and Cacoyianni 1990). Within laboratory experiments where aggressive interactions amongst *D. melanogaster* males are observed, it is typical that larger males or males that hold residence of a territory first, have greater reproductive success (Hoffmann 1987). Considering this, if populations are within an environment that allows males to benefit from territorial behavior, these individuals may show an increase in fitness, and more variation in mating strategies. Yet to date, most experimental evolution and purging experiments have not considered these explicit factors in their design.

While the previous work outlined above has made considerable contributions to our understanding of the interplay between environmental complexity and selective forces, the environments used in these experiments are relatively simplistic when considering the plasticity of animal mating behavior. We conducted a series of short-term allele purging experimental evolution assays where environmental complexity and the accessibility of *D. melanogaster* to critical resources were manipulated with these factors in mind. In the first experiment we looked at how variation in resource patch size and accessibility influenced purging of six recessive deleterious mutant alleles from populations being held within a series of complex environments. Specifically, we provided multiple resource patches of high (to maximize female fecundity) and low quality. In each treatment high quality patch size and accessibility varied according to how they should potentially influence aspects of territoriality. In the second experiment, we examined how the rate of removal for two of these mutant alleles differed between the complex environments and two simple environments in which we manipulated opportunity for mate choice (via forced monogamy). We expected that if natural and sexual selection were aligned, we would see an increase in purging rate as accessibility to resources decreased as individuals that display territorial behavior are more likely to obtain mates within these environments and thus there is a selection for "good genes." We also predicted that the purging rate overall would be greater when sexual selection was allowed to act in the form of mate choice than when it was removed.

Methods

ENVIRONMENTAL MANIPULATIONS

Images of the environmental treatments and an illustration of general set up are provided in Figure 1. Three environments were created in order to test the effects of desirable resource availability on the removal of deleterious mutations from populations. Within each environment there were both "high quality" resources of a yeast-rich food (see Table S1) and a dilution to 15% (in water/carrageenan) of this food as a "low quality" resource. High quality food was determined based on previously published nutritional geometry studies (Lee et al. 2008; Jensen et al. 2015), that maximized female fecundity. The intent of the high quality food resource was to entice females to use patches for oviposition and potentially lead males to defend these resources to maximize their mating success. The 15% diluted medium was developed (based on pilot experiments) to be sufficient to support adult survival, but where larval developmental time increased and viability decreased. Eggs laid on the low quality resource would be unlikely to contribute to the next generation, as few emerged, and those that eclosed from it did so after adults were collected for the next generation. As such adults were not competing for resource patches for survival *per se*, but for the desirable resources that females may prefer to maximize their reproduction. In our design, total selection is a combination of sexual, fecundity and viability selection, although our experimental design does not allow us to disentangle them.

For each replicate environment described below, mesh BugDorm4M1515 cages (15 cm^3) were used. The "non-territory" treatment environment (NT) consisted of a single *Drosophila* culture bottle (177 mL), with a surface area of 30.25 cm^2 (55 mm × 55 mm base) containing approximately 50 mL of high quality food with the addition of four drops of a yeast-paste and orange juice mixture on top (to attract females (Dweck et al. 2013)), as well as a bottle with 50ml low quality food. These represent "typical" *Drosophila* lab environments where apparent scramble competition is commonly observed (Spieth 1974), although subtle interference competition may be occurring as well (Baxter et al. 2018). The "unconstrained territory" spatial treatment (UCT) consisted of eight open vials (height of 50 mm, 25 mm diameter, 4.9 cm^2 surface area) each filled with approximately 5 mL of high quality food with a single drop of yeast-paste/orange juice mixture on top and a single bottle with the low quality food. Finally, the "spatially constrained territory" treatment (SCT) had the same set-up as the UCT treatment except

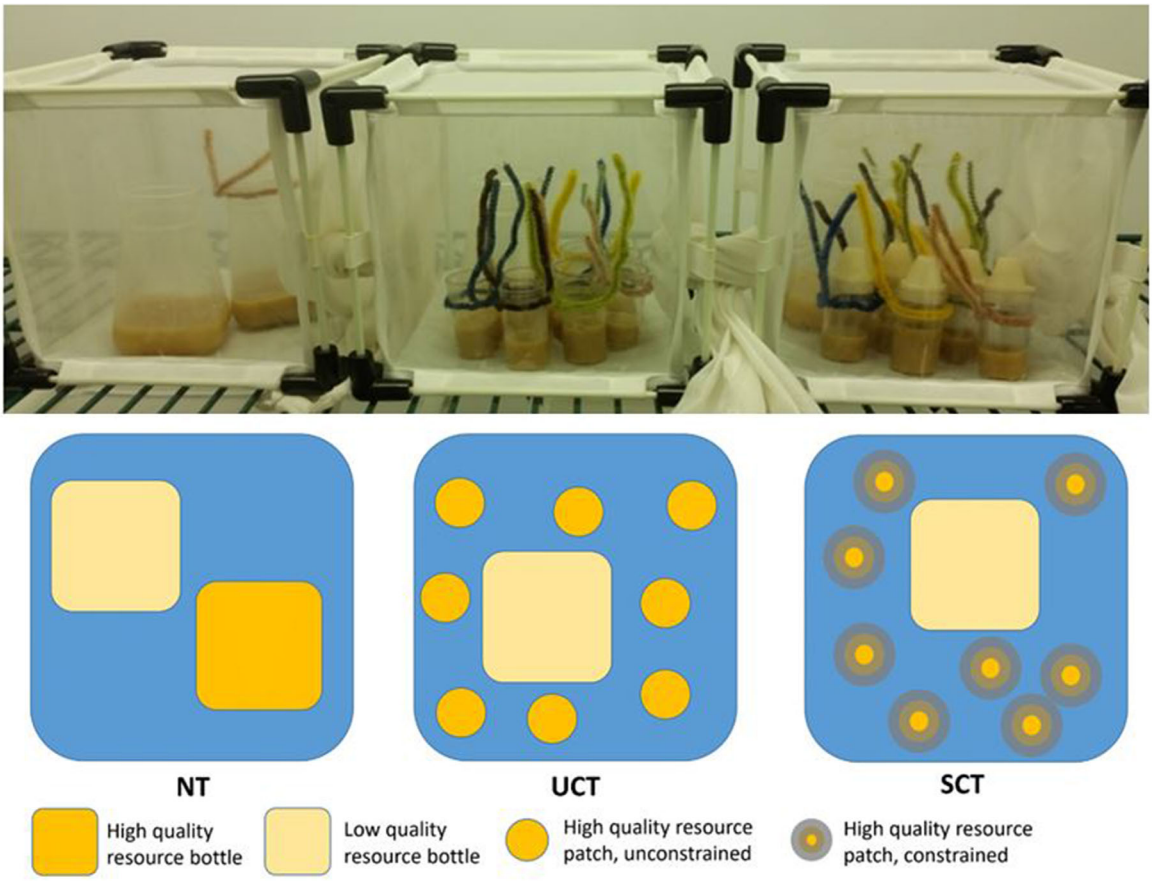

Figure 1. Top: Environmental treatment set up for non-territory (NT - left), unconstrained territory (UCT - middle), and spatially constrained territories (SCT - right). Bottom: Overhead schematic of environmental treatment set up. See Figure S1 for detailed schematic of the 3D printed cap used in the SCT treatment.

each vial had a 3D printed cap (22 mm diameter, 25 mm height, 4 mm opening, see Figure S1) to further restrict ease of access to high quality food patches. These 3D printed caps were designed and tested with several specific features in mind. First, that it was relatively difficult to gain access, but would be relatively easy (given positive photo-taxis and negative geo-taxis in *Drosophila* (Markow and Merriam 1977)) for an interloper to be chased out. Second, that the aperture was of sufficient size that two large *D. melanogaster* individuals could pass one another, but one individual could still harass or chase the other in this space. Finally, the cap was designed so that if an individual did display territorial behaviors, it had multiple places to survey or defend (food surface, inner aperture, and outside top of aperture). Pipe cleaners were wrapped around the tops of bottles and vials to provide additional perching substrate for individuals.

POPULATIONS

To examine deleterious allele purging rates, six mutations with known morphological defects were used across each of the three spatial treatments. Each allele was picked because of previous work examining the effects of selection on them in the context of either spatial manipulations or varying degrees of sexual selection (Arbuthnott and Rundle 2012; Colpitts et al. 2017). Three of these mutations are autosomal (*brown*^{1}, *vestigial*^{1}, and *plexus*^{1}) and three are X-linked (*white*^{1}, *yellow*^{1}, and *forked*^{1}). The mutations *plexus*^{1}, *white*^{1}, *yellow*^{1}, and *forked*^{1} were obtained from Bloomington stock center while *brown*^{1} and *vestigial*^{1} were obtained from stocks kept in the lab. These alleles were chosen for their wide array of phenotypic effects with two influencing eye colour (*white*^{1} and *brown*^{1}), two influencing wing morphology (*plexus*^{1} and *vestigial*^{1}), one affecting body colour and behaviour (*yellow*^{1}) and one affecting bristle morphology (*forked*^{1}).

To create experimental populations, individuals were backcrossed into a large outbred domesticated lab population (census size of 1200–1600 individuals) originally collected from Fenn Valley Winery (FVW), Michigan (GPS co-ordinates: 42.578919, −86.144936) in 2010. This population was chosen to minimize confounding effects of lab adaptation in this experiment (Harshman and Hoffmann 2000), that is, it is expected that this population has already had considerable opportunity to adapt to

our lab environment (∼180 generations prior to initiation of this experiment). To generate experimental populations, the following procedure was used. For autosomal mutant alleles, mutant female virgins were crossed with FVW males. F1's were then crossed to each other and mutant homozygote females were collected. For X-linked mutant alleles, hemizygous mutant males were crossed with wildtype females. The heterozygous females from this cross were then crossed back to wildtype males, the mutant offspring from this cross were then collected and the process was repeated. For each mutant allele, backcrossing was conducted for five cycles and on the final generation, offspring from the final cross were mated together to create mutant males and females. Fifty pairs were used to generate each cross.

PURGING RATES ACROSS ENVIRONMENTS

For each mutant allele, nine replicate populations were created and three of each randomly assigned to one of the three environmental treatments. Initial populations consisted of 100 males and 100 females with starting allele frequencies of 0.7 for their respective mutant allele. Populations were maintained at 12L:12D cycles at 21°C with 60% relative humidity in a Conviron walk in chamber (CMP6050). Each generation, adults were placed into their respective treatments and allowed to mate and lay eggs for three days. After which adults were removed from the environments and discarded. Eggs were allowed to develop for 11 days, after which the next generation of adults was collected by bringing the adults to the cold room kept at 4°C and gently knocking them into vials. After this initial collection, 100 males and 100 females from each replicate were phenotyped under light CO_2 and placed into their respective environments with fresh food. This cycle was repeated for 10 generations.

Due to a laboratory bacterial infection in one replicate of the *brown*^{1} population for the NT treatment, this replicate was discarded after generation 5. A fourth replicate was created with the same starting allele frequencies (0.7) in order to account for the missing data. This replicate was therefore five generations behind the rest of the experiment and was continued for 10 generations.

In order to get an estimate of allele frequencies for autosomal mutant alleles during this experiment, monogamous pairings of phenotypically wildtype females and mutant males were conducted at generations 3 and 6, for *brown*^{1} and *plexus*^{1} populations and at generations 3 and 8 for *vestigial*^{1} populations. After the collection of adults for the next generation, for each population 50 virgin females were phenotyped over light CO_2. Of the 50 females, those that lacked the mutation (i.e., could be homozygous or heterozygous for the wild-type alleles) were placed singularly into vials with a mutant male. Offspring were analyzed from these vials over 3 days after initial emergence of offspring. If a vial contained only wildtype offspring, the female parent was scored as homozygous for the wild type allele, if the vial contained a mixture of wildtype and mutant offspring, the female parent was scored as heterozygous for the mutant allele. For X-linked mutant alleles, allele frequencies were estimated from the frequency of the mutation in males.

PURGING RATES WITH EFFECTS OF MATE CHOICE

To determine the effects of sexual selection on purging rates, we re-ran the experiment using *white*^{1} and *vestigial*^{1} with the addition of two new treatments. The allele *vestigial*^{1} was chosen to further examine the drastic purging rate seen within the first few generations of the initial experiment, also as this mutant allele affects wing development and subsequently mobility and courtship signalling (Pezzoli et al. 1986), it was expected to be under strong natural and sexual selection. The allele *white*^{1} was chosen for further investigation because according to the genotypic frequencies from the initial experiment, it followed the expected pattern of the SCT treatment having the greatest purging rate, and due to its' use in previous allele purging studies, could be used as a comparison of our work to others (MacLellan et al. 2009; Arbuthnott and Rundle 2012; Colpitts et al. 2017). The first treatment, "vial no choice" (VNC), consisted of randomly assigning 100 individual pairs into vials to mate (i.e., forced monogamy). The second treatment, "vial choice" (VC), consisted of randomly assigning 100 male and female adults into vials of five mixed sex pairs. After three days of mating for each treatment, males were removed and females were placed into environments similar to the NT treatment. After three days, females were removed and eggs allowed to develop for 9–10 days. Emerging female virgins and adult males were collected similar to above and the process was repeated. NT, UCT, and SCT treatments were conducted the same as above except females were collected as virgins and males and females were held separately for three days after collection in order to align with the experimental schedule of the VNC and VC treatments. This experiment was conducted for only four generations as it was disrupted by a lab shutdown brought about by the COVID-19 pandemic. One replicate of the SCT *vestigial*^{1} treatment did not have any surviving adults at generation four.

STATISTICAL ANALYSIS

The rate of mutant allele loss in each population over multiple generations for each component of the experiment was analyzed by fitting generalized linear mixed effect models with binomial distribution and a logit link (i.e., a logistic mixed model). Since each allele was started at a known frequency, and the intercept was known, models were fit without estimating a global intercept (but included offsets). Main effect for allele or treatment were also not included (as all treatments started with the same frequency for a given allele). Fixed effects included in the model

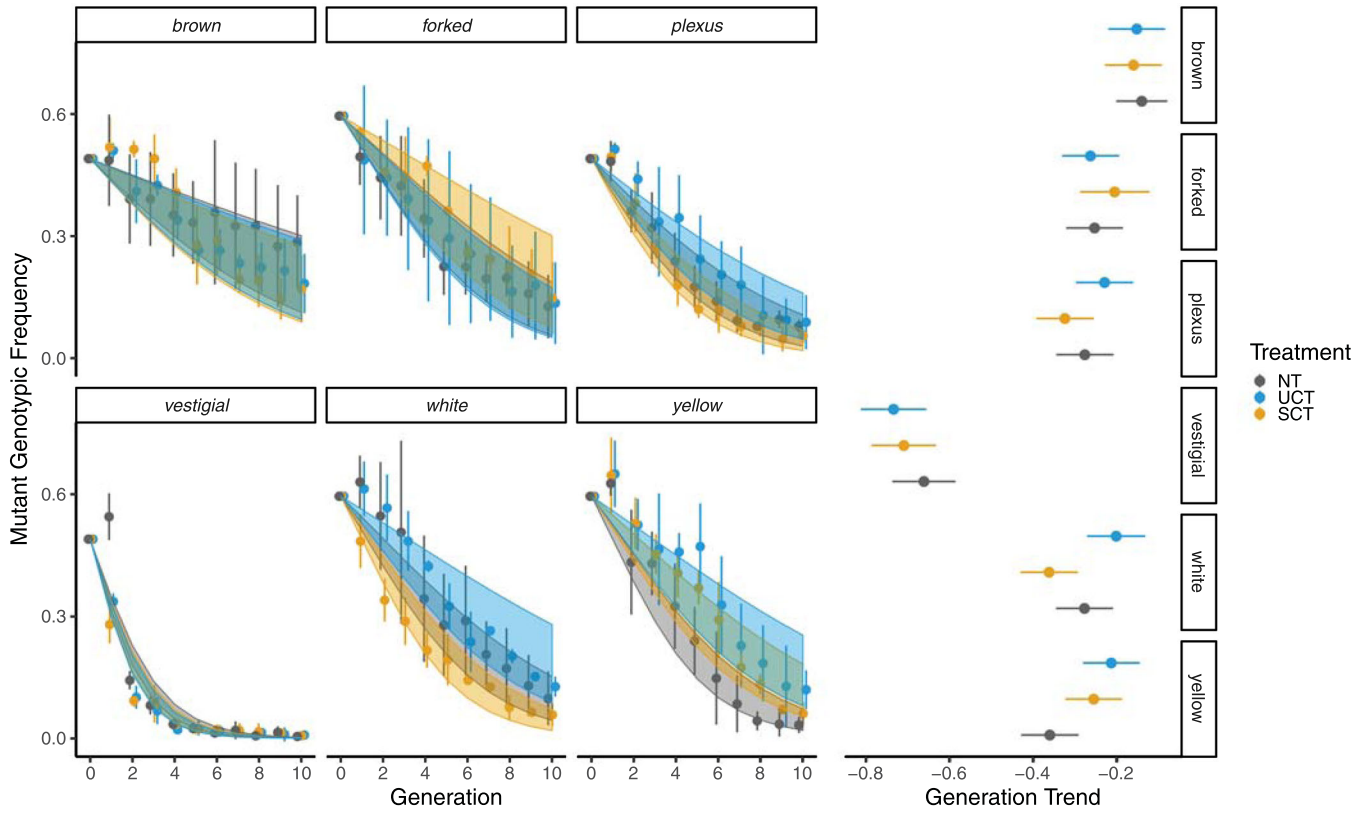

Figure 2. **Left: Purging rates across the three environmental treatments for each mutant allele. Data points and error bars represent mean mutant genotypic frequencies and standard deviations across three replicates. Confidence bands represent 95% confidence intervals for our generalized linear mixed model. Right: Average generational effects for each mutant allele based on model estimates. Error bars represent 95% confidence intervals.**

were thus generation X mutant allele, generation X treatment, and generation X mutant allele X treatment. Random slopes for generation were fit across replicate lineages, and the intercept was offset to 0.7 for allele frequency (or 0.49 for autosomal and 0.595 for sex-linked mutations when modelling mutant genotypic frequencies). Fixed effects were further examined for significance with a two way ANOVA (type II Wald χ^2 test) and treatment contrasts averaged over mutant allele were examined by comparing estimated marginal means within each model. Contrasts were calculated using emmeans and Tukey's HSD to adjust for number of comparisons.

For analyzing purging rates across environmental treatments, models were generated with and without the third SCT replicate for the *forked*[1] mutation due to this replicate having mutant allele frequencies approaching fixation consistently throughout the experiment (Figure S2, Tables S2 and S3). Results presented exclude this replicate unless otherwise indicated.

Selection coefficients for each mutant allele were estimated from allele frequency data using the generalized linear mixed effect model, computing the average slope for each mutant allele and treatment across each generation. This is analogous to computing $log(\frac{p}{1-p})$ for each generation and calculating the slope to estimate s, but allows us to use information across the whole model.

All statistical analyses were performed in R version 3.5.2 (R Core Team 2018) using glmer() (lme4 package version 1.1-21; Bates et al. 2015), Anova() (car package version 3.0-2; Fox and Weisberg 2011), and emtrends() (emmeans package version 3.1.1; Lenth 2019). All plots were generated with ggplot2 v.3.1.1 (Wickham 2016).

Results

As expected, average allele frequency declined over the ten generations of experimental evolution for all six mutant alleles, consistent with the alleles being deleterious (Figure 2). We observed substantial differences in rates of purging (as assessed by genotypic frequencies) across the six mutant alleles. The ANOVA shows significant interaction effects of generation with mutant allele and treatment, however significant effects may be restricted to certain mutant alleles as contrast estimates between treatments

Table 1. ANOVA for fixed effects from four generalized linear mixed models produced from the six mutant alleles across the three treatment types.

Both sexes		χ^2	df	P
	Generation × treatment	66.13	3	**$2.88e^{-14}$**
	Generation × mutant allele	380.81	5	**$2.20\ e^{-16}$**
	Generation × treatment × mutant allele	22.05	10	**0.02**
Males only				
	Generation × treatment	79.57	3	**$2.20e^{-16}$**
	Generation × mutant allele	476.51	5	**$2.20e^{-16}$**
	Generation × treatment × mutant allele	24.69	10	**0.006**
Females only				
	Generation × treatment	36.42	3	**$6.09e^{-8}$**
	Generation × mutant allele	194.25	5	**$2.20e^{-16}$**
	Generation × treatment × mutant allele	24.69	10	**0.006**
Allele Frequency				
	Generation × treatment	17.98	3	**0.0004**
	Generation × mutant allele	80.53	5	**$6.49e^{-16}$**
	Generation × treatment × mutant allele	16.59	10	0.08

Table 2. Estimates and significance of treatment contrasts among the six mutation alleles for four generalized linear mixed models.

Both Sexes	Contrast	Estimate	P
	NT – SCT	0.008	0.92
	NT – UCT	−0.029	0.33
	SCT – UCT	−0.037	0.18
Males only			
	NT – SCT	0.014	0.74
	NT – UCT	−0.024	0.40
	SCT – UCT	−0.038	0.12
Females only			
	NT – SCT	−0.004	0.99
	NT – UCT	−0.050	0.20
	SCT – UCT	−0.045	0.28
Allele Frequency			
	NT – SCT	−0.008	0.93
	NT – UCT	−0.010	0.88
	SCT – UCT	−0.003	0.99

among all mutant alleles are non-significant (Table 1 and Table 2). Across the six mutant alleles, there was no consistent overall pattern in purging rate between NT, UCT, and SCT environmental treatments. Similar results are shown when analyzing males and females separately. When examining estimated allele frequencies, only the interactions between generation and mutant allele, and generation and treatment are significant (Figure 3, Table 1). However, treatment contrasts are still not significantly different from one another (Table 2). Overall trends from the ANOVA and treatment contrasts are similar when including the third SCT replicate for the *forked*[1] mutation. Estimated selection coefficients differ substantially by mutant allele, however these also indicate no consistent pattern in strength of selection of treatment types across mutant alleles (Figure 3). Overall, the results suggest that while there are effects of the three spatial treatments on rates of purging (Figure S3), they are relatively modest in comparison to the effects of individual mutant alleles and their interactions with spatial treatment. We tested for differences between observed and expected genotypic frequencies as a coarse assessment of assortative mating within genotypes (Table S4), but observed no evidence of significant deviation after correcting for multiple comparisons.

In the second experiment, we replicated the first allele purging experiment with two mutant alleles and added additional treatments with explicit manipulations of sexual selection. The removal of sexual selection for both *white*[1] and *vestigial*[1] mutant populations decreased purging rates (Figure 4, Table 3). While the forced monogamy treatment (VNC) showed the slowest purging rate for both mutations, between the treatments that include sexual selection there is no consistent pattern in purging rate by treatment across the two mutant alleles. The ANOVA shows significant effects of the interaction between generation and mutant allele, and generation and treatment but not for the interaction between all three fixed effects. Treatment contrasts show that the VNC (vial no choice) treatment (i.e., forced monogamy) is significantly different from the other treatments but VC, NT, UCT, and SCT are not significantly different from each other. When analyzing the sexes separately, only the interactions between generation and treatment, and generation and mutant allele were significant for males whereas the interactions between generation

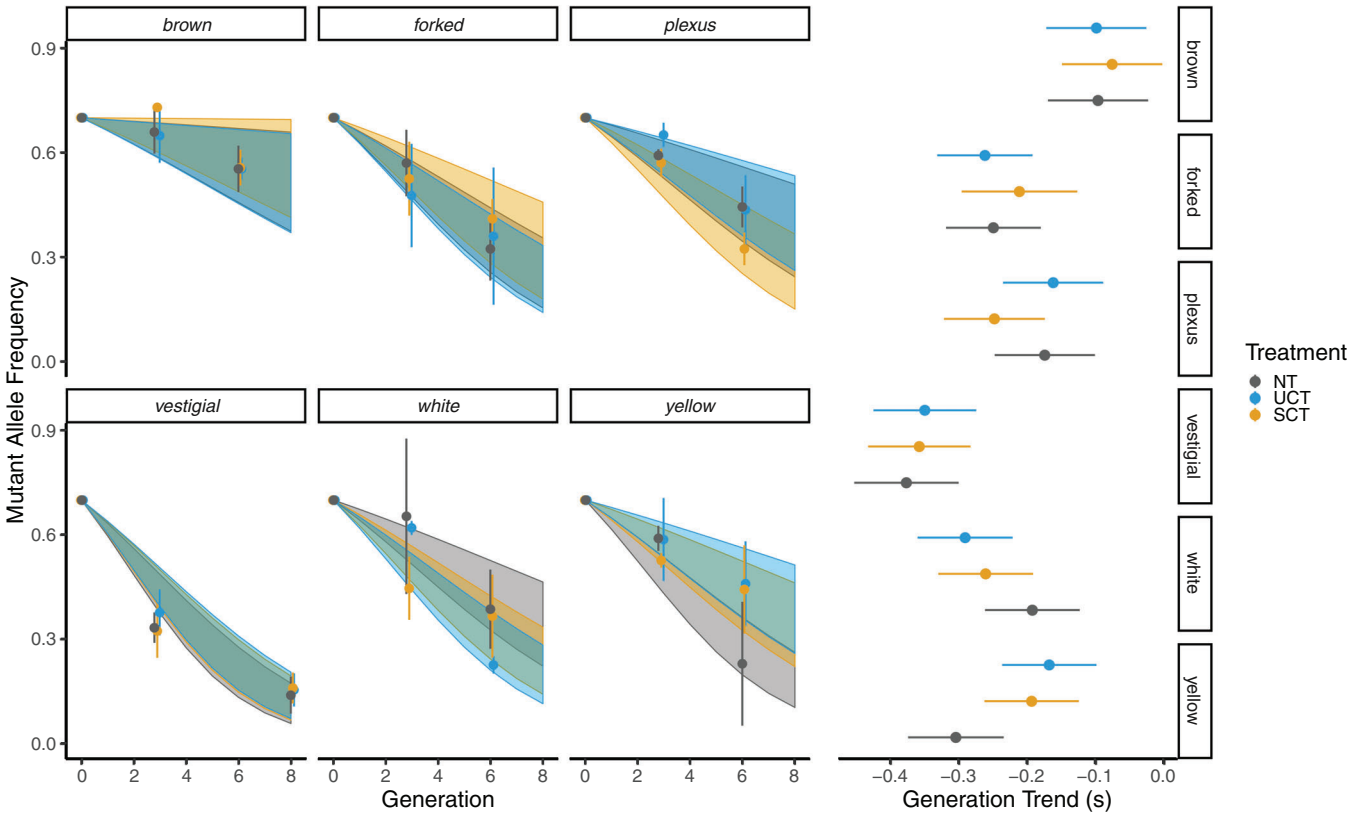

Figure 3. Left: Purging rates across the three environmental treatments for each mutant allele. Data points and error bars represent mean allele frequencies and standard deviations across the three replicates. Confidence bands represent 95% confidence intervals for our generalized linear mixed model. Right: Estimated selection coefficients (s) for each mutant allele based on model estimates. Error bars represent 95% confidence intervals.

Table 3. ANOVA for fixed effects for three generalized linear mixed models produced from the two mutant alleles across the five treatment types.

Both sexes		χ^2	df	P
	Generation × treatment	973.56	5	**$2.00e^{-16}$**
	Generation × mutant allele	97.78	1	**$2.00e^{-16}$**
	Generation × treatment × mutant allele	5.21	4	0.27
Males only				
	Generation × treatment	1272.95	5	**$2.00e^{-16}$**
	Generation × mutant allele	460.20	1	**$2.00e^{-16}$**
	Generation × treatment × mutant allele	8.85	4	0.07
Females only				
	Generation × treatment	431.31	5	**$2.00e^{-16}$**
	Generation × mutant allele	1.03	1	0.31
	Generation × treatment × mutant allele	12.39	4	**0.02**

and treatment, and generation, treatment, and mutant allele were significant for females. Treatment contrasts were similar between male and female models with only the VNC treatment showing a significant difference from other treatment types when looking across all mutant alleles (Table 4).

Discussion

Spatial heterogeneity in the environment can alter many aspects of an organisms' phenotype including mating strategy. In turn, this influences how selection acts on a population, including the degree to which allelic effects may be concordant or antagonistic

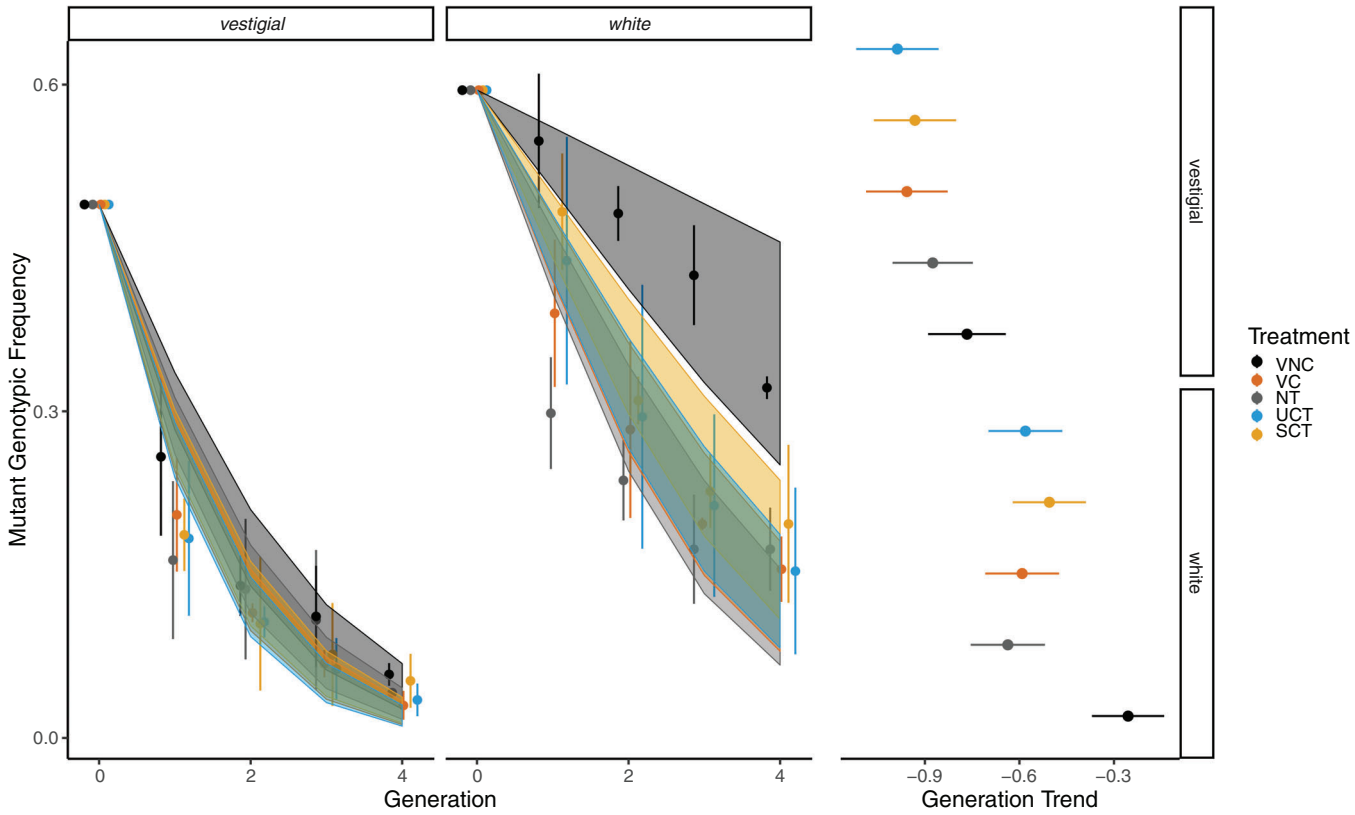

Figure 4. **Left: Purging rates across the five environmental treatments for each mutant allele. Data points and error bars represent mean mutant genotypic frequencies and standard deviations across the three replicates. Confidence bands represent 95% confidence intervals for our generalized linear mixed model. Right: Average generational effects for each mutant allele based on model estimates. Error bars represent 95% confidence intervals.**

across fitness components. Analyzing the directions and magnitudes of components of natural selection has been investigated in many contexts, however many empirical studies teasing apart these elements in varying environments fail to recognise the influence of mating strategies. We created populations with specific deleterious mutant alleles segregating at high frequencies. We allowed these populations to evolve in environments differing in spatial constrains for resource accessibility to determine how environmental complexity influences the removal of deleterious mutations. We observed environmental complexity did influence purging rates, but these depended greatly on specific deleterious alleles. We performed a second experiment examining purging rates of two mutant alleles in the same environments including additional treatments allowing different opportunities for mate choice within a "simple" environment. Again, we found that purging rates between treatments varied with mutant allele. For both alleles, a lack of mate choice through forced monogamy – substantially reducing opportunity for sexual selection – decreased purging rates compared to the treatment where mate choice was present.

For each of the six mutant alleles, we expected that with increased variance in resource accessibility there would be an increase in purging rate and therefore the highest purging rate would be seen in the SCT treatment, with the lowest being in the NT treatment. This prediction rested on several assumptions including that natural and sexual selection are aligned, high quality food patches in the UCT and SCT treatments would initiate territorial behavior within males, and males of the highest quality would be able to hold and defend these food patches with the most success, leading to the most mates. While the SCT treatment showed the highest purging rate among treatment types for *plexus*[1] populations, this pattern does not hold for other mutant alleles. This discrepancy between our predictions and the data could be due to inaccurate assumptions or other unknown factors. Despite evidence that *Drosophila melanogaster* among other *Drosophila* species can show context dependent territoriality (Dow and von Schilcher 1975; Hoffmann 1987, 1988; Hoffmann and Cacoyianni 1990; Chen et al. 2002; Saltz and Foley 2011; Baxter et al. 2015), considerable uncertainty exists in the extent of what factors influence it and how it ultimately influences the fitness of an individual.

Table 4. Estimates and significance of treatment contrasts among *white*[1] and *vestigial*[1] mutations from three generalized linear mixed models.

Both Sexes	Contrast	Estimate	P
	VNC – NT	0.245	**0.0007**
	VNC – VC	0.263	**0.0002**
	VNC – SCT	0.207	**0.007**
	VNC – UCT	0.273	**0.0001**
	NT – VC	0.018	1.00
	NT – SCT	−0.038	0.98
	NT – UCT	0.028	0.99
	VC – SCT	−0.056	0.90
	VC – UCT	0.010	1.00
	SCT – UCT	0.066	0.84
Males Only			
	VNC – NT	0.214	**0.001**
	VNC – VC	0.339	**<0.0001**
	VNC – SCT	0.171	**0.02**
	VNC – UCT	0.284	**<0.0001**
	NT – VC	0.125	0.27
	NT – SCT	−0.043	0.95
	NT – UCT	0.070	0.77
	VC – SCT	−0.168	0.06
	VC – UCT	−0.055	0.92
	SCT – UCT	0.113	0.35
Females Only			
	VNC – NT	0.356	**0.0001**
	VNC – VC	0.339	**0.0003**
	VNC – SCT	0.301	**0.002**
	VNC – UCT	0.350	**0.0002**
	NT – VC	−0.016	1.00
	NT – SCT	−0.055	0.97
	NT – UCT	−0.006	1.00
	VC – SCT	−0.039	0.99
	VC – UCT	0.011	1.00
	SCT – UCT	0.049	0.98

We discuss this issue more fully below in the "caveats" section. It should also be noted that evolutionary stable strategy theories predict that a behavioral strategy will only be adopted by an individual or population if it is advantageous (Maynard Smith 1974). While our environments were designed based on theory that would suggest our assumptions provide the most advantageous strategy (Emlen and Oring 1977; Emlen 2014), this cannot be known without further empirical testing and observation and other strategies may have been implemented that cause the discrepancy between our expectations and results.

An additional potential explanation for the heterogeneity in results is that the relative contribution of sexual, fecundity and viability to total selection varied by individual allele. In turn these effects potentially interacted with the different spatial environmental treatments. In those cases where total selection was dominated by viability selection, any influence that our treatments had (influencing sexual and fecundity selection) would be more difficult to observe. This would be similar to previous findings that partitioned sexual and non-sexual components of fitness against deleterious alleles while varying population density (Sharp and Agrawal 2008). Our first experiment was not designed to explicitly partition sexual, fecundity, and viability selection. However, our second experiment clearly demonstrated sexual selection had a substantial impact on purging rate, and thus total selection. This is consistent with previous work (Sharp and Agrawal 2008).

Although our results do not show any consistent pattern of purging rate across treatment types between mutant alleles, heterogeneous results are common to many purging experiments. Many find that each mutation acts differently in experimental treatments not only in magnitude but also direction and thus mainly focus on the overall patterns among mutation alleles (Sharp and Agrawal 2008; MacLellan et al. 2009; Arbuthnott and Rundle 2012; Clark et al. 2012; Maclellan et al. 2012; Colpitts et al. 2017; Singh et al. 2017). These differences are also reflected in the estimated selection coefficients, where the environmental treatment that has the highest selection coefficient changes depending on mutant allele. Differences between how these mutant individuals interact within their environment may explain these variances. For example, the mutant *vestigial*^{1} has reduced wings necessary for movement and courtship signalling (Pezzoli et al. 1986), putting it at a greater disadvantage compared to wild-type individuals in the same population. This is likely why it has the most drastic purging rate across environmental treatments among all the mutations analyzed in this study. Further investigation into the behaviors of these mutant alleles may give an indication as to why these results differ between mutant alleles. Indeed, the X-linked *yellow*^{1} mutation used in this study has been widely used previously because of reduced competitive ability of y^{1} males in acquiring mates. This is in part because wild type females have reduced preference for y^{1} males (Barker 1962; Liu et al. 2019) in addition to reduced mating speeds, potentially mediated by defects in the structural integrity of male sex combs (Massey et al. 2019). A purging experiment with y^{1} designed to partition total selection demonstrated that while viability selection did contribute, much of the selection on this allele was due to sexual selection (Liu et al. 2019).

While we wanted to explore how resource accessibility and environmental complexity influence populations through purging rates, we also wanted to evaluate how these compared to the purging rates of populations that lacked sexual selection and populations that had simple mating environments. As expected, removal of sexual selection decreased purging rates for both mutant alleles. However, there was no difference between the simple and relatively complex environments in purging rate for

either mutant allele. This contradicts previous work of Colpitts et al. (2017) where polygamous populations of mutant *white*^{1} *D. melanogaster* showed increased purging rates in complex environments. While the overall ideas between our experiments are similar, key differences in experimental design could explain these inconsistencies. First, due to the alignment of the experimental schedule, virgins from the VNC and VC treatments were able to mate more quickly than the virgins in the NT, UCT, and SCT treatments that were initially held separately before mating. This difference in waiting times to mate could have caused virgins from the NT, UCT, and SCT treatments to be more receptive to potential mates (Pavković-Lučić and Kekić 2009). This could also explain why we see differences in the overall trends between the NT, UCT, and SCT environments compared to our initial experiment. Second, our experiment had a much shorter mating period (3 days versus 6) and all eggs laid during this time period were kept to potentially contribute to the next generation for the NT, UCT, and SCT treatments, but not for the VNC and VC treatments. This could potentially lead to lower quality offspring from early matings with lower quality males being kept within the experiment, decreasing the purging rates within the complex mating treatments.

CAVEATS

To avoid confounding effects of an interaction between lab adaptation (domestication) with the purging of deleterious alleles we explicitly chose a population that already had more than 180 generations to adapt to the lab environment. However, genetic variation for mating strategies and optimization of using the spatial environments provided could have been reduced in this population due to lab adaptation or genetic drift. However, we do not think this is likely as lab adapted populations (including this one) show tremendous amounts of genetic and phenotypic variation, including for behavioral traits such as aggression. One must also consider whether any of the mutant alleles might influence the degree of assortative mating occurring in these populations. While there is no consistent evidence for such effects in the literature for most deleterious alleles, wild type females prefer wild type males in comparison to y^{1} males, but y^{1} females show relatively weak preferences (Barker 1962). As a partial check for this (but which is confounded by the strength of selection) was a comparison between observed and expected genotypic frequencies (Table S4). However, we observed no evidence for deviations in expected genotypic frequencies.

An additional caveat to this experiment is that, although mating strategies and behaviors were intended to be analyzed directly within these environments, these experiments could not be conducted due to reduced lab access arising from complications from the COVID-19 pandemic. The NT treatment was designed to resemble environments that promote commonly observed scramble competition in *Drosophila melanogaster*. The common conditions used in order to increase territoriality in both the UCT and SCT treatments have been shown to increase the rate of territorial behavior and the success of those males that defend territories (Hoffmann and Cacoyianni 1990). There have been a wide array of studies using this reduced resource patch size in order to demonstrate the presence of and the details of aggression and territoriality in *Drosophila* (Dow and von Schilcher 1975; Hoffmann 1987, 1988; Chen et al. 2002; Saltz and Foley 2011; Baxter et al. 2015). While many of these studies are based on short term experiments, it has recently been shown that in natural environments male aggression can persist for longer time periods (Dukas 2020) like those in the current experiments. The one condition which we were unable to evaluate experimentally was whether the addition of the cap (SCT treatment), increased the efficacy in which males holding a territory could chase away interlopers. In other words, whether SCT increases the resource holding potential of the males beyond that of UCT. However, this does not diminish our results when comparing the NT environment to the UCT and SCT environments, which are based on numerous previous behavioral studies. Therefore, our study still demonstrates the influence of spatial heterogeneity on mating strategy and the importance of its' consideration when analyzing components of natural selection. Overall, our study adds to the recently growing body of literature considering "environmental complexity," while breaking down "complexity" further to accommodate for changes in mating strategy by environment.

AUTHOR CONTRIBUTIONS

AW substantial contribution to design, acquisition of data, analysis, interpretation of data, and drafting of manuscript. A.S. substantial contribution to acquisition of data. I.D. substantial contribution to design, analysis, interpretation of data, and drafting of manuscript.

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DATA ARCHIVING

Data and scripts are available on github (https://github.com/idworkin/Wilson2021_Evolution_Data) and Dryad (https://doi.org/10.5061/dryad.m37pvmd24).

CONFLICT OF INTEREST

The authors confirm they have no conflict of interest.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1: Recipe for high quality food and nutritional content.
Table S2: ANOVA for fixed effects of four generalized linear mixed models produced from the six mutant alleles across the three treatment types including all replicates.
Table S4: Observed genotypes from each cross used to estimate allele frequencies with expected values in parentheses.
Figure S1: Schematic for 3D-printed cap design. Caps were created using filament material.
Figure S2: Left: Purging rates for the *forked1* mutant allele while including all replicates. Data points and error bars represent average mutant genotypic frequencies or allele frequencies and the standard deviations across all replicates.
Figure S3: Left: Purging rates across the three environmental treatments averaging across mutant alleles. Data points and error bars represent mean mutant frequencies and standard deviations across the three replicates of each mutant allele.