Sex in a virtual reality: experimental evidence for sexual isolation due to variation in perception of the environment

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Abstract

Differential perception and subsequent differential use of habitats can generate local adaptation, especially when natural selection cannot. However, this local adaptation is not maintained into future generations unless mating happens within the chosen habitats. We currently have no experimental data on whether differential perception of environments results in sexual isolation. We induced differential perception of environments by stimulating different olfactory neurons via light pulses (optogenetics) in two groups of fruit flies. These flies were released in a cage of which only one section received light pulses. One group of flies perceives this optogenetic stimulation as the smell of a harmful concentration of CO2 and was found to avoid the illuminated section. The other group perceives it as the smell of food-related compounds and was found to be attracted to the illuminated section. Due to this self-imposed spatial segregation, we subsequently observed a considerable degree of sexual isolation between the two groups of flies. In contrast, in two control treatments preventing differential perception of the environment, sexual isolation was virtually absent. Our results show that differential perception of the environment can easily and rapidly generate spatial segregation and sexual isolation among individuals that are ecologically different. This can maintain local adaptation, especially under conditions when natural selection cannot, which are increasingly common due to human-induced rapid environmental change.

INTRODUCTION

Environments vary in space and time, and organisms can adapt in different ways to this variation[1,2]. Natural selection is a frequent driver of adaptive evolution in natural populations. However, its ability to do so is often limited. For example, evolutionary responses will lag behind when environments change quickly, and homogenizing gene flow will limit local adaptation when environments vary over small spatial scales[3]. These limitations to natural selection are increasingly relevant as human activities induce rapid environmental change and create novel environments[4,5], requiring populations to adapt or face extinction.

An alternative route to local adaptation is matching habitat choice. Here, individuals assess their ecological performance across available habitats and make greater use of those habitats perceived to allow a better performance. In effect, gene flow is non-random[6] with respect to performance and does not erode but enhances population divergence in the ecological traits underlying that performance. Matching habitat choice can thus result in rapid local adaptation at very small spatial and temporal scales[3,7–11] . However, maintaining the achieved local adaptation into the next generation requires that mating happens between individuals that prefer the same local habitat – because if individuals mate randomly across habitats, all local adaptation is lost[12]. In contrast, when mating is local, matching habitat choice improves individual and population fitness across generations, colonization of novel habitats is promoted, genetic variation is maintained, different locally adaptive traits have a larger probability of recombining into even more locally adapted genotypes, and it may even provide the initial steps towards speciation[8,12–19]. Mating within the chosen habitat therefore has crucial consequences. The resulting assortative mating for locally adaptive traits

is predicted or assumed by several theoretical studies[12,18–21]. However, so far there are no experimental tests of whether variation in the perception of the environment results in assortative mating.

To remedy this, in this study we measured the strength of assortative mating when variation in the perception of the environment was manipulated experimentally. For this we used optogenetics[22], a tool developed in neurobiology to remotely control the activity of neurons using light. As a novel application of this technique in ecology and evolution, we manipulated the olfactory neurons of two groups of *Drosophila melanogaster*which changed their perception of their environment. In response to light exposure, one group of flies was designed to smell food (a positive stimulus), while the other group was designed to smell a dangerously high concentration of CO_2 (a negative stimulus). We combined both groups of flies, exposed them to a habitat lit by red light and another habitat that was not, and allowed them to choose between these. (Hence, the flies live - and have sex - in a virtual reality, as in the movie Inception). We then measured the degree of assortative mating between the two types of flies due to any spatial population structure induced by the flies themselves. To control for any assortative mating due to other reasons, we also measured assortative mating when the heterogeneity of the environment could not be perceived by the flies (the optogenetic neurons were inactivated; see Methods), and when the environment was actually homogeneous.

RESULTS

We confirmed that habitat choice depended on variation in perception of the environment. In accordance with the predictions, flies perceiving smell of CO_2 were repelled by exposure to light and mostly moved to the dark habitat, while flies perceiving smell of food were attracted to the illuminated habitat (supplementary notes and Fig. S1). This differential perception of the available habitats and subsequent spatial sorting resulted in sexual isolation. The Pair Sexual Isolation (PSI) statistic compares the observed frequency of a specific mating pair to the frequency expected under random mating[23,24]. When optogenetic stimulation was activated and two different habitats were available (treatment 2), same-genotype pairs were more frequent than expected by chance alone (Fig. 1). In contrast, when optogenetic stimulation was inactivated (treatment 1) or the habitat was homogeneous (treatment 3), median PSI values did not deviate from random mating.

Figure 1. Box plots of Pair Sexual Isolation (PSI) indices for the 4 possible pairs in each replicate across treatments. 'A' refers to flies carrying the Gr21a driver (positive stimulus-sensing), and 'B' refers to flies carrying Or42b (negative stimulus-sensing). Higher PSI scores indicate a higher probability of observing a certain pair: a PSI of 1 is expected under random mating (dotted line). Treatment 2 (grey-filled boxes) exposes optogenetic flies to heterogeneity in light, and therefore to differential perception of the environment. In control treatment 1 the flies are not optogenetically enabled (i.e., they were not fed ATR, see methods), so they cannot perceive the heterogeneity. In control treatment 3 the environment is homogeneous (i.e., there are no red lights present).

The I_{PSI} index[25] combines all PSI values of a population into a single estimate of assortative mating. This index takes a value between -1 and 1 and is negative when it is disassortative and positive when mating is assortative (a value of 1 means all gene flow is interrupted). We found that for treatment 2 the I_{PSI} scores were always positive (mean = 0.50), while the scores for control treatments 1 and 3 were distributed around 0 (mean = 0.04 and 0.03, respectively; Fig. 2). A comparison of the I_{PSI} scores using pair-wise non-parametric Wilcoxon tests yielded significant differences between treatment 2 and control treatments 1 and 3 ($Z=3.061$, $p=0.002$ and $Z=4.052$, $p<0.001$, respectively), but not between treatments 1 and 3 ($Z=0.269$, $p=0.788$).

Figure 2. The I_{PSI} index for each replicate. This score is a combination of all four PSI scores (see Fig. 1). The exclusively positive values for treatment 2 (grey-filled distribution) mean a consistent occurrence of positive assortative mating under differential perception of the environment, while in treatments 1 and 3 the values are balanced around 0 (random mating). Treatment 2 exposes optogenetic flies to heterogeneity in light. In control treatment 1 the flies are not optogenetically enabled, so they cannot perceive the heterogeneity. In control treatment 3 the environment is homogeneous.

DISCUSSION

Habitat choice evolves as a mechanism to increase the match between environmental conditions and organismal traits[1,16,26]. This is expected to improve the ecological performance of the individual, to result in the local adaptation of populations, and to prevent local and global extinction[1,8,10,15,27]. While these are important effects, they are shared with other processes that can increase phenotype-environment match and expected fitness, such as phenotypic plasticity or niche construction[1,28]. However, habitat choice has an effect that these alternative processes do not have: it structures individuals in space, which increases the likelihood of assortative mating among individuals with comparable ecological traits, without the need for mate choice[12,19]. To the extent that these ecological traits are heritable, this will result in the production of locally adapted offspring, and better performance of the population in that habitat.

These effects of habitat choice are well-known[10,12,15,16,18,19,29–31]. However, there are three different kinds of habitat choice[8,16], and their effects are not the same. When habitat choice is based on a genetically encoded or an imprinted preference for a specific habitat, all the effects mentioned above are rather indirect: they only arise if there is a correlation between preference and ecological traits. This means that when the correlation between preference and ecological traits is weaker, the effects of habitat choice are also weaker. And when there are disruptions in the relationships between phenotype, environment, and fitness, genetically encoded or imprinting-based habitat choice can cause the emergence of ecological traps[32,33]. Matching habitat choice does not suffer from these weaknesses, since preference is directly based on ecological performance, and not on preference-trait correlations that only indirectly (and potentially poorly) predict fitness[9,10,12,19,34]. This is an advantage of matching habitat choice under human-induced rapid environmental change, as it might even operate beneficially in habitats never before encountered during the species' evolutionary history.

Matching habitat choice should be able to increase assortative mating for local ecological performance if mating occurs in the chosen habitat. The ability of matching habitat choice to drive assortative mating has been repeatedly discussed in the theoretical literature[6,8,12,14,15,18,19], yet so far there is hardly any experimental evidence to support it (but see[35,36]). We here show that fruit flies choose habitats depending on variation in their perception of ecological performance (see supplementary notes), and that this results in a fairly strong degree of assortative mating among individuals with the same perception (Fig. 1 and 2). In our two control treatments (where all the flies are functionally the same, or the two habitats are the same), no such assortative mating is seen. This indicates that the differential perception of expected performance in each environment (a key component of matching habitat choice) was responsible for the observed assortative mating, i.e., flies preferred different parts of the cage and then mated locally. This result confirms the assumptions in the literature on matching habitat choice, thereby increasing the relevance of its theoretical predictions discussed above. It also shows the importance of how organisms perceive their environments for small-scale population structuring and sexual isolation.

An interesting comparison with the concept of "magic trait" can be made. A magic trait is a trait that is exposed to divergent natural selection and also happens to cause assortative mating[12,20,37]. With matching habitat choice, any and all ecological traits causing variation in local performance across environments can promote assortative mating as an indirect effect of causing spatial clustering. Matching habitat choice is therefore acting more like a "magic process" enabling the origin and operation of magic traits. Both the relevant ecological trait(s) and the capacity for matching habitat choice need to evolve for this. This is greatly facilitated by the fact that the same alleles coding for matching habitat choice are favored across all environments, and hence no genetic polymorphism is required to originate and be maintained in linkage disequilibrium with the ecological traits (*i.e.* , population divergence involves a one-allele mechanism, cf.[38]). In situations where random gene flow would otherwise prevent divergence, matching habitat choice is therefore a much more likely driver of adaptive population divergence than habitat choice due to genetic preference alleles, which does require a polymorphism maintained in genetic disequilibrium with ecological traits[8,9,12,15]. It is also a much more likely driver than mate choice, which requires similarly demanding coupling between mating traits, mating preferences, and ecological traits (reviewed in[12]). In our scenario involving matching habitat choice, the genetical and evolutionary complications of mate choice are avoided: assortative mating is simply an incidental (yet powerful) by-product of spatial grouping for perceived ecological performance.

We made use of optogenetics[22]. This modern technique has been developed in neurobiology for applications like mapping the function of specific neurons[39,40]. It also allows for the manipulation of behavior[41–43]. However, as far as we know, it has not been used before to test for the ecological and evolutionary consequences of behavioral variation. Optogenetics allows for very specific and very rapid neural control. Given the large range of behaviors that can be manipulated optogenetically in different organisms (including some vertebrates), we expect this technique to be employed more often in ecological and evolutionary studies.

Forced exposure to the optogenetic stimuli had no significant effect on survival or fecundity of our flies (see supplementary notes). Therefore, it might be argued that the flies in our experiment did not make decisions based on *actual* ecological performance and expected fitness. However, input obtained from sensory neurons is perceived as real by these flies when assessing habitat quality[44–46]. Hence, avoiding the perceived CO_2 is in principle an adaptive response of the fly in their virtual reality, even if CO_2 is not present in their actual reality. Similarly, movement towards food odors is in principle adaptive, but all flies were tested with *ad libitum*food already. The upshot of this lack of effects of optogenetic stimulation on realized fitness is that we can exclude disruptive natural selection across the two experimental habitats as an alternative explanation for the observed assortative mating.

Therefore, our study confirms that adaptive population divergence (and perhaps even the initiation of speciation) is not only driven by differential birth and death, *i.e.* , natural selection. It can also be driven by differential immigration and emigration, *i.e.* , non-random gene flow[1,2,6,8,12,14,15,18,19,47]. Unfortunately, because other mechanisms of adaptation can result in comparable phenotype-environment match in nature, matching habitat choice is relatively hard to identify in the wild[27,35,48–55]. Most of these studies are not conclusive about the mechanism driving adaptation and divergence, but here we have shown conclusively how differential perception of habitats - the key component of matching habitat choice - can cause population divergence and sexual isolation.

Matching habitat choice is expected to be transient, and to be replaced by other mechanisms of adaptation[1,28]. This presumably leads to an underappreciation of its potential to drive the adaptation and divergence of populations, especially at small spatial and temporal scales where adaptive divergence due to natural selection is hampered[3]. Due to local and rapid environmental change driven by human actions, matching habitat choice might both be easier to detect nowadays, and increasingly important for population survival.

METHODS

In this study we used optogenetics to manipulate how *D. melanogaster* fruit flies perceive the quality of two different sections of their living space. We tested whether differential perception of the environment can produce assortative mating between two groups of otherwise identical individuals in a habitat without any physical barriers.

The experimental set-up consisted of a transparent acrylic cage in which the flies were released, placed inside an opaque box that blocked light from the outside (Fig. 1). Two of the inward-facing sides of the box were covered by an array of red LEDs (625 nm) that provided an irradiance of 8.5 W/m^2. An opaque cover was placed over one half of the inner cage to create a dark environment (0.2 W/m^2). Red light is virtually invisible to the flies[56,57] which reduces undesired effects due to visual stimulation.

Figure 3. Experimental arena. a) The outer cage with the lights used for optogenetic stimulation. b) The inner cage where the flies are released, including a petri dish with food on each end of the long side. An opaque cover blocks the light from entering one half of the cage. During the experiments, these arenas were covered by an opaque lid.

Our experimental populations are composed of two kinds of flies in terms of their perception of the illuminated part of the habitat. Half of the flies perceive optogenetic stimulation as the smell of a harmful concentration of CO_2[58–61] and are expected to avoid the illuminated habitat. The other half of the flies perceive it as the smell of food-related compounds[46,59,60,62] and are expected to be attracted to the illuminated habitat.

Fly stocks, husbandry, and rearing

The repellent stimulus was induced using a Gr21a-GAL4 optogenetic driver[44,60,63] (Bloomington fly strain #23890). The attractive stimulus was induced using an Or42b-GAL4 driver[46,60,62,64] (Bloomington strain #9971). We used the responder UAS-Chrimson[39] (Bloomington fly strain #55136). The optogenetic flies were produced by crossing flies from either of the two driver strains with flies of the responder strain. Before eclosion, pupae were sexed by identifying the sex combs on the first pair of legs of the males. This method ensures virginity with minimum handling and anesthesia. The hatched flies were tested 3 or 4 days after eclosion.

To infer which males sire the observed offspring, in each replicate the males of one of the strains presented the *Tubby* phenotype. *Tubby* is a dominant and visible mutation[65](*Dmel\Tb* mutation Tb^1, FBgn0243586 in FlyBase) that causes larvae, pupae and, to some extent, adults to be thicker and shorter than *wild type* . We alternated the genotype of the *Tubby* -carrying male between replicates, to balance out the potential effects of expressing the mutation. The optogenetic *Tubby* flies were produced by replacing the UAS-Chrimson strain with a UAS-Chrimson strain that carries the balancer chromosome TM6B (Bloomington fly strain #81080), which contains the*Tubby* mutation[66] .

All the stocks were maintained on conventional wheat flour, yeast, and sugar medium at 25 °C. While most strains are reared under a 12h/12h light-dark cycle, optogenetic flies were kept in the dark, to avoid accidental optogenetic responses to ambient light. To enable the activation of the light-sensitive ion channels, the food for optogenetic flies contained a 0.5mM concentration of *all-trans* -retinal (ATR).

Assortative mating assays

In preparation for the assays, flies fasted for 24 hours inside a Petri dish with a small piece of paper soaked in water to prevent dehydration. In the case of optogenetic-enabled flies, this water had a 0.5mM concentration of ATR to retain optogenetic activity. To distinguish between different optogenetic genotypes, females were put to sleep with a brief exposure to CO_2 and a small spot was painted on one of the wings of each female with an alcohol-based permanent marker, one color for each genotype.

Two Petri dishes were prepared for each replicate: one containing the two kinds of females (Gr21a and Or42b) and another one containing the two kinds of males (Gr21a and Or42b, one of them marked with*Tubby*). 10 flies of each sex/kind combination were used. At the beginning of each replicate, the Petri dishes containing the flies were placed inside the arena, in the central section of the inner cage. Each dish had a piece of thread attached to the lid that protruded outside of the arena. Pulling this thread removed the lid and released the flies. To force the starved flies to explore, 1ml of food was placed on either end of the arena. Females were released immediately after the cage was closed and were allowed to explore for four hours before releasing the males. This prevented flies from engaging in courtship before having had a chance to explore, compare and choose their habitat. (This would not be an issue if we had let the hatched flies mature in the experimental set-up (as would be the case in nature), but that would have taken excessively much time, and we would not have been able to mark the female genotypes). 20 hours after the males were released, the females were taken out of the experiment and each one put in a separate vial containing standard food and left to lay eggs for 48 hours in the dark. Once all the larvae had developed into pupae, we counted the number of*Tubby* and *wild type* pupae produced by each female.

Data analysis

We measured the degree of sexual isolation for each replicate using the I_{PSI} index[25]. The absolute value of this index (between 0 and 1) indicates the strength of assortative mating in the population, while its sign indicates whether it is assortative (+) or disassortative (-). This is obtained from the Pair Sexual Isolation (PSI) coefficients that compare the observed frequency of mating pairs to what is expected under random mating[23,24]. Females that produced any*Tubby* -marked offspring were considered to have mated with the*Tubby* -marked male genotype. Re-mating was a possibility and there is no obvious way to distinguish between females that were fertilized by males of both genotypes and those that only mated with*Tubby* -marked males. The effect

of remating was compensated for by alternating which male genotype carried the *Tubby* marker. Our simulations (see supplementary notes and Fig. S2) show that while the PSI of pairs containing a *Tubby* -marked male was overestimated, the combined I_{PSI} can be reliably inferred. We conservatively tested for differences in I_{PSI} values between treatments using pair-wise non-parametric Wilcoxon tests, because it is not clear if the I_{PSI} values are expected to adhere to any standard statistical distribution.

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