Dissecting yeast-dependent population differentiation and spatial segregation in Drosophila melanogaster
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Chapter 6

Adaptation to different food resources plays an important role in the diversification of phenotypes and species. Characteristic examples are seen from herbivorous insects (Dres and Mallet, 2002; Funk et al., 2002; Matsubayashi et al., 2010). For instance, the Enchenopa treehopper populations living on different host plants that differ in phenology have different timings of egg hatch and thereby asynchronous mating windows, which promotes assortative mating between these populations (Wood and Keese, 1990). Specialization on conifers by the aphid genus Cinara, which mates on its host plants including Abies, Cedrus, Picea and Pinus, leads to host plant-associated reproductive isolation and species formation in this genus (Jousselin et al., 2013). In this thesis, I start by formulating seven key questions that can be used to understand the processes leading up to food-mediated ecological speciation based on the knowledge gaps in the nature of the heterogeneity exerting divergent selection, the molecular basis of chemosensory adaptations to different food resources and the characterization of behavioural responses contributing to reproductive isolation. In the following chapters of my thesis, rather than aiming to examine all these seven questions, I focus on using Drosophila melanogaster as the model organism to experimentally investigate two questions regarding food exploitation and food-mediated reproductive isolation that are critical for leading up to food-mediated speciation. I choose D. melanogaster as the model organism because a significant body of knowledge regarding different aspects of fly biology and ecology, the numerous assays for testing food-dependent life-history traits and well-studied associations between D. melanogaster and its food source-yeast are available in D. melanogaster. In addition, D. melanogaster represents an imperfect generalist state between its seasonal specialist ancestor and its highly specialist relatives (e.g., Drosophila sechellia) and is thereby an informative model organism for studying the early stages of dietary specialization and food-dependent differentiation.

I experimentally investigated which trait allowed the consumer to successfully exploit the food resource in Chapter 3. I found that D. melanogaster could feed, grow, reproduce and survive on all the yeast species we tested, but different life-history traits were maximized on different yeast species. This indicates that all the traits we tested allow D. melanogaster to exploit yeast but the total trait performances across different life-stages may limit the exploitation of yeast. This is seen in the observation that overall fitness values on different yeast species were all in-between the hypothetical worst and best. With the calculated fitness values on different yeast species, I explored another mechanism limiting food exploitation - fitness trade-offs, which have been the most frequently investigated and cited explanation for the evolution of dietary specialization in herbivorous insects (Futuyma and Moreno, 1988; Hardy et al., 2020). Previous field and experimental studies on insect–plant interactions provide strong evidence that there are genetic constraints on the evolution of dietary breadth (reviewed in Hardy et al., 2020). Antagonistic pleiotropy, where an allele that is favoured by one food source is unfavoured on another, can affect food-dependent phenotypes and result in fitness trade-offs (Hardy et al., 2020). I do not detect trade-offs between different yeast species. Fly performance tends to be positively rather than negatively correlated across yeast species, suggesting there are no genetic constraints limiting the utilization of different yeast
species. This absence of trade-offs together with the lack of the alignment between different life-history traits is in line with the generalist strategy *D. melanogaster* maintains throughout most of its global distribution. Notably, for the calculation of overall fitness values, I simply treated each trait equally and summed each standardized trait value without taking the relative contribution of each trait to fitness into consideration since I did not want to make too many assumptions. However, some life-history traits may be more strongly linked to overall fitness than others. For instance, survival rate seems more critical than developmental rate or mating rate for fitness. Different weights can be assigned to different life-history traits and may thereby affect the observed overall fitness values and the pattern of how these values lie in with respect to the hypothetical worst and best and the fitness trade-offs. Together, I may conclude that researchers should analyse a suite of life-history traits involved in food exploitation to explore the scope of food exploitation and dietary specialization.

I investigated one of the various routes to reproductive isolation between individuals that adapted to an alternative food resource – assortative mating in Chapter 4 & 5. Different food resources can drive assortative mating through its spatial coupling with mates as assortative mating can arise as a by-product of food choice when mating takes place on food resources. I did not directly test assortative mating between multiple *D. melanogaster* strains and different yeast species as all the fly strains I tested prefer the same yeast, but the results regarding the effects of light and time on mating location provide important implications for the possible situation in nature. For instance, temporal isolation may coincide with the spatial segregations between populations that prefer alternative food resources. *D. melanogaster* under laboratory condition exhibits a bimodal locomotor activity profile and is thus crepuscular, but wild populations display high variation of being diurnal or nocturnal (Pegoraro et al., 2020). When populations go to different food resources at different times with respect to the light cycle and mate there, they can become reproductively isolated from each other both in space and time. In this scenario, assortative mating can be facilitated by the effects of light and time on mating location.

**Dietary adaptation & Preference-performance relationship**

The hypothesis that females maximize their fitness by ovipositing on plants where their offspring will perform the best, has been proposed as an important evolutionary outcome of dietary adaptation in herbivore insects (Jaenike, 1978; Craig and Itami, 2008). This hypothesis suggests a correlation between adult female preference and offspring performance (e.g., growth, survival and reproduction) and receives support from many insect studies (Craig and Itami, 2008). In Chapter 3, I focused on testing the performance instead of preference of different fly strains on different yeast species since I aim to explore how flies perform on different yeast species and whether these performances are correlated across traits. In addition, performance rather than preference can be a proxy of fitness. Therefore, I did not directly test any preference in Chapter 3. But I tested the mating and oviposition preference of one lab strain *Canton-S* between two yeast species *Candida boidinii* and *Candida californica* in Chapter 5. Combining the preference data in Chapter 5 and the performance data in Chapter 3, I find that *Canton-S* prefers *C. boidinii* over *C. californica* for oviposition
(Figure 5, Chapter 5) but it takes longer for eggs to pupate on *C. boidinii* compared with *C. californica* (Figure S 2, Chapter 3), suggesting a negative correlation between adult female preference and offspring development. Notably, adult Canton-S were tested with killed yeast for preference in Chapter 5 but live yeast for performance in Chapter 3. As killed yeast loses its fermenting capacity and mainly functioning as a protein source (Grangeteau et al., 2018) and egg-laying preference is strongly influenced by yeast fermentation products (Joseph et al., 2009), the oviposition preference may change when flies are exposed to choices of live yeast. The negative correlation between adult female preference and offspring development may then disappear or become totally different. Therefore, this thesis cannot provide direct experimental evidence regarding whether adult female preference is positively correlated with offspring development for maximal fitness. Yet, this thesis suggests that females do not know the best yeast for their offspring.

**Complexity of yeast-dependent differentiation in *D. melanogaster* in nature**

In this thesis, I focus on the heterogeneity that yeast provides and take this as a starting point to understand whether yeast diversity, together with the global distribution of *D. melanogaster*, might promote the adaptive divergence of different strains and induce assortative mating in *D. melanogaster*. Specifically, I tested the biological interactions between yeast species and *D. melanogaster* strains. In nature, the ecological interactions between *Drosophila* and yeast can be more complex compared with laboratory conditions.

Yeast does not exist alone in the field. It grows on substrates including fruits, flowers and mushrooms. In addition to the variation in yeast itself (e.g., fermentation capability, physiological growth profiles and the production of metabolites), flies may also experience the consequences of variation in the substrates where yeast grows, the distribution of these substrates and the fermentation profiles of yeast on these different substrates. Furthermore, it is more a microbial community than a single yeast species that serves as the feeding niche for flies (Chandler et al., 2012). Yeast occurs in communities with several yeast species and other microbes like bacteria on different substrates. The yeast-yeast and yeast-bacteria interactions may also affect fly biology. For example, flies reared with a mixture of two yeast species obtain higher fitness with higher viability and shorter development time compared to monocultures (Starmer and Aberdeen, 1990). The mixed microbial community composed of yeast and bacteria is more attractive to flies than individual members because of the emergent metabolites from the yeast-bacteria interactions (Fischer et al., 2017). Therefore, flies experience more complexities in the heterogeneity of yeast in ecological settings compared to laboratory settings. The effects of yeast on fly life history and spatial allocation in nature will depend on multiple variables including the substrates where yeast grows, the fermentation profiles of yeast on these substrates and the interactions between yeast and other microbes.

In addition, yeast is not only present in the substrate as a food source, but also survives on the fly body and in the fly digestive tract as a symbiont. In adult flies, yeast cells are transported through the fly body (mainly through the abdomen) to new substrates and persist.
in the fly digestive tract (Christiaens et al., 2014; Hoang et al., 2015). Yeast cells can also get horizontally transferred to the opposite sex during courtship and mating (Starmer et al., 1988). In *Drosophila* larvae, live yeast cells pass through the digestive tract and are excreted in faecal pools (Stamps et al., 2012). Through these ways, yeast is vectored by flies and flies can actively carry yeast to new environments. Therefore, the yeast species which flies experience in their environment may include the yeast resident in the fruits and the yeast flies carry. The symbiont yeast species together with the bacteria flies carry form the microbial symbionts in flies. These microbial symbionts can not only affect host fitness like development, fecundity and lifespan but also the mate choice of hosts (Sharon et al., 2011; Gould et al., 2018; Heys et al., 2018). Consequently, these microbial symbionts may influence the dynamics of yeast-dependent differentiation either through their effects on the host or through their interactions with the yeast which flies feed on. Thus, the effects of microbial symbionts on the dynamics of yeast-dependent differentiation can be a promising direction for understanding the scope of yeast-dependent differentiation.

Taken together, yeast-dependent differentiation in *D. melanogaster* in nature is a complex process that depends on multiple factors including the variation in the substrates where yeast grows, the fermentation profiles of yeast on these different substrates, the interaction between yeast and other microbes in the substrates and the microbial symbionts flies carry. I thereby conclude that further studies that consider multiple interacting partners are needed to understand yeast-dependent differentiation in *D. melanogaster* in nature.

**Concluding remarks**

This thesis starts with seven key questions that can be used to understand how the chemosensory system can facilitate food-mediated ecological speciation and then focuses on experimentally investigating two main questions – food-mediated population differentiation and assortative mating. Using *D. melanogaster* and its food source yeast as a model system, I quantify multiple yeast-dependent life history traits across life stages to explore on the scope for dietary specialization and population differentiation (Chapter 3) and track the mating location of flies in environments containing heterogeneous food patches to explore the sensory and behavioural mechanisms underlying the co-localization of food and mates and the potential of assortative mating based on dietary divergence (Chapter 5). Each chapter provides some new insights. I find that the inconsistency of responses between life-history traits is a possible route limiting dietary specialization and that food preference may directly lead to assortative mating through its spatial coupling with mates. My findings inspire several specific avenues for future research on understanding the mechanisms of diet evolution and food-mediated assortative mating. I list four main research opportunities which may contribute to exploring the mechanisms.

**Future work**

1. Will fly strains reared on different yeast species differentiate from each other?
In Chapter 3, I evaluated the scope for yeast-dependent population differentiation when I expose fly strains to different yeast species and directly test fly performances on these yeasts. Together with other findings that adult food choice and mating behaviour as well as longevity were significantly influenced by the yeast species added in fly juvenile diet (Murgier et al., 2019), I therefore hypothesize that rearing flies on different live yeast species will affect yeast-dependent life-history traits and thereby yeast-dependent differentiation. With the extensive screen on the interactions between fly strains and yeast species in Chapter 3, potential combinations of fly strains and yeast species can be chosen to do the following long-term evolutionary experiment to explore the occurrence of yeast-dependent differentiation. The possible fly-yeast combinations can be selected from the significant interactions in different life-history traits as we observed in Chapter 3. By rearing fly strains on live yeast and testing trans-generation effects of yeast, we can explore the evolution of yeast-dependent differentiation.

2. Will dietary generalists and specialists differ in the inconsistency between trait responses across life-history traits?

Based on the results from Chapter 3, I hypothesize that the lack of alignment across yeast-dependent life history traits may limit *D. melanogaster* dietary specialization and is thus a possible explanation for the limited extent of dietary specialization in *D. melanogaster*. I thereby predict that whether trait responses are inconsistent between life-history traits can be where dietary generalists and specialists differ. Therefore, one promising direction for a deep understanding of diet evolution can be examining traits responses to specific diets in dietary specialists and comparing the pattern between generalists and specialists. I propose *D. sechellia* as the possible candidate specialist which can be tested and compared with *D. melanogaster*.

3. Will individuals preferring alternative and spatially separated yeast species become spatially segregated and reproductively isolated?

In Chapter 5, I find female-male pairs specifically go to yeast for mating after virginal mating and prefer mating on one yeast over another in *D. melanogaster*. These results suggest there is scope for yeast-mediated assortative mating. Based on these results, I hypothesize that flies which prefer alternative and spatially separated yeast species will become spatially segregated and reproductively isolated. Thus, future research on testing two fly strains and two yeast species simultaneously in a cage or a room experiment can be illuminating for understanding the occurrence and the condition of yeast-mediated assortative mating.

4. Will the aggregation pheromones play a potential role in yeast-mediated spatial segregation?

In *D. melanogaster*, food odours can stimulate males to deposit a pheromone that guides the aggregation of both sexes (Lin et al., 2015). Substrates with aggregation pheromone are significantly more attractive compared with pheromone-free substrates (Wertheim et al., 2006). This inspires me to ask if there will be a synergetic effect between yeast and
aggregation pheromones in modulating fly location and thereby affecting the potential of yeast-mediated spatial segregation. For instance, will flies prefer an unattractive yeast species with aggregation pheromones over an attractive yeast without such pheromones as a mating location? Further studies on integrating aggregation pheromones into understanding spatial distribution and mating location of fly pairs will generate important insights on yeast-mediated spatial segregation and assortative mating.