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A Review of Pollinator Interactions and Their Role in Shaping Plant Hybrid Zones

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ABSTRACT

The interactions of plants and pollinators within hybrid zones is the focus of this review paper, with an emphasis placed on how such interactions promote speciation events and evolutionary change. Pollinators affect the spread of genetic material, shown using examples such as Orchis, Joshua Trees, and penstemon hybrid flowers. It has been historically challenging to create accurate hybrid zones maps, especially because their boundaries rapidly adjust due to climate change, habitat loss, and pure chance. Creating accurate maps is further complicated due to the large amounts of genetic material within the hybrid zones themselves. The importance of pollinator interactions for plant reproduction is described, discussing how reproductive barriers can impact plant speciation. Examples of coevolution between plants and pollinators is used to further explain the mutual importance they have on one another. Despite current understanding, further research on hybrid zones is still needed to explain factors that promote speciation within hybrid zones. Due to newly improved DNA sequencing and analysis technologies, scientists can create maps of hybrid zones more accurately and with less funding. Not only can these technologies expand current understanding on why speciation occurs within hybrid zones, but can also suggest strategies to conserve biodiversity.

Introgression and Its Evolutionary Implications

Exploring Evolutionary Dynamics

Understanding how species change their genotypes over time is a principle idea of evolutionary biology. The ways different plant species interact with one another in hybrid zones, and how pollinators influence these interactions represents a key focus within the field, as these interactions have the opportunity to promote new species emerging. Hybrid zones are areas where genetically different organisms are able to come together to produce an offspring. They are locations where scientists can study processes such as gene flow, selection, and speciation (Barton & Hewitt 1989). Through studying hybrid zones, scientists can better understand what evolution is, how it occurs, and why interactions between plants and pollinators promote evolutionary change. The first theme of this review paper is to discuss the exchanges of genetic material that happen in hybrid zones, and how these exchanges influence evolution, new traits, and new species emerging (Hewitt 1988).

The second main theme of this review paper are the relationships between plants and pollinators. Within hybrid zones, pollinators, such as bees, transport the genes of plants (through pollen) to nearby plant populations (Walton et al. 2020). Because plants have little capability to spread their pollen across far distances themselves, they rely on pollinators for this spread. To further explain the importance of pollinators for plant reproduction, examples where pollinators have directly influenced the emergence of new plant species are used.

Due to recent advancements in DNA analysis, the final theme of this paper are the ways improvements in technology change the way scientists study hybrid zones. Microorganisms and other species that have been historically challenging to analyze can now be studied via DNA sampling, improving our understanding of hybrid zone interactions.

Hybrid Zones

New research is still being conducted to increase our understanding of the genetic processes that occur in hybrid zones. The genomes of different species can intertwine, allowing for large amounts of gene flow within these hybrid zones (Pickup et al. 2019). Introgression, the transfer of genetic material from one population to another, can lead to the emergence of new species (Grant & Grant 2014). Hybrid zones are locations where introgression commonly occurs. However, it is important to understand that not all hybrid zones will lead to introgression due to factors such as sexual selection of species (Yang et al. 2020).

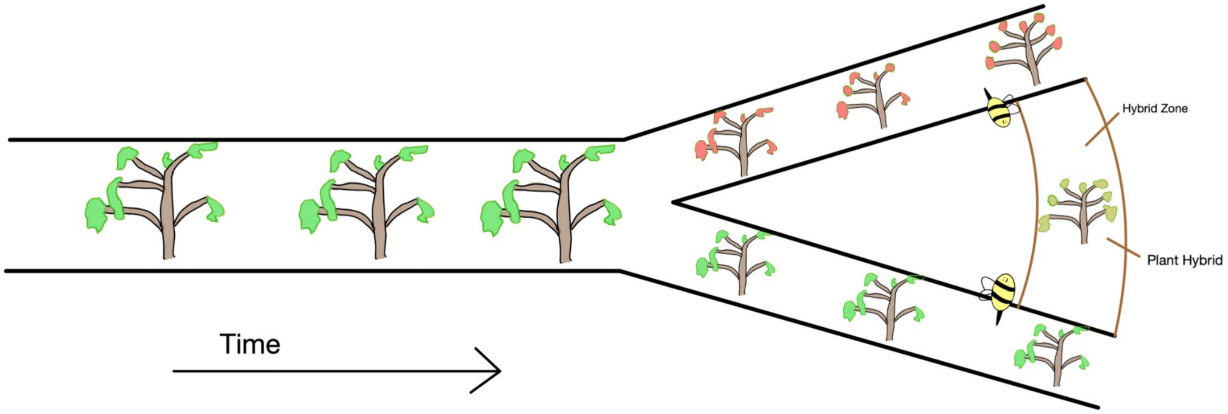

Figure 1. Formation of a Plant Hybrid Zone and the Role of Pollinators. The native plant population has green leaves, shown on the left side of the figure. Due to a barrier in gene flow, a different plant population with red leaves emerged with a different leaf shape. The area where the native green plant and red plant populations come into contact is a hybrid zone, where hybridization between the two occurs. The bees shown in the figure transfer pollen between the green and red populations, promoting cross breading and the creation of the hybrid plant.

The impacts that introgression has within hybrid zones cannot be overlooked. Gene flow through introgression heavily contributes to the emergence of new traits (Newhouse & Powell 2021). Hybrid zones allow different species to exchange genetic material via cross fertilization. For example, data collected from four related species of Orchis suggest that hybridization occurred in the past, creating different species of Orchis due to plant and pollinator interactions. Despite strong postzygotic isolation, all the Orchis have characteristics in common and share a similar geographic location. It is concluded Orchis populations have never been made up of hybrid populations within these hybrid zones entirely. Instead, hybridization previously occurred, and continues to occur today (Bersweden et al. 2021). The evolution of Orchis stresses the importance of pollinators, especially in hybrid zones. Multiple evolutionary processes, such as natural selection, genetic drift, and reproductive isolation are able to lead to new species emerging and influence plant / pollinator interactions. For example, not all pollinators transport pollen between the same species of plants. The pollinator's choice of plant is commonly measured in terms of consistency and preference. Consistency is when common plants are able to spread their pollen to other common plants at higher rates than rare plants. Contrary, preference is when pollinators visit a certain plant species more often, causing reproductive isolation and potentially new species (Hopkins 2022). Preference of a pollinator can be in terms of flower color, shape, size, or amounts of nectar. Plants which pollinators prefer will have their pollen transferred across other plants from that same species at higher rates, leading to possible reproductive isolation and the formation of hybrid zones over time (Hopkins 2022).

Despite research on the impacts hybrid zones have on speciation, it remains a challenge to map hybrid zones and estimate distances between them. The large variety of genetic material within these regions complicates the process further, making it increasingly difficult to tell the difference between parent species and hybrids (Rieseberg & Buerkle 2002). It also remains a challenge to map the outer boundaries of hybrid zones because many times there is a sudden decrease in population towards these hybrid boundaries, complicating the border drawing process, and affecting the way hybrid zone maps show species overlap (Taylor et al. 2015).

External factors, most notably climate change, have the potential to change the structure of hybrid zones in a relatively short time. Hybrid zones are able to shift due to various factors, outlined by the tension zone model (Taylor et al. 2015). Although changes in hybrid zone location are often due to humans through habitat destruction or introduction of non-native species, it is important to understand that in many cases, changes in hybrid zones can be due to chance alone (Taylor et al. 2015). It remains a limitation to identify the cause for such a shift in hybrid zones, whether being due to humans, climate change, environmental conditions, or a variety of all. Thus, it is important to be able to sample populations in order to accurately map out hybrid zones, especially ones that take up large areas (Taylor et al. 2015). By studying the movement of hybrid zones over time, not only can a deeper understanding on plant and pollinator interactions be inferred, but information can be gathered on climate change and how species adjust to it.

Studying hybrid zones and their distributions can suggest conservation strategies to preserve biodiversity and protect vulnerable populations (Whitham et al. 1999). This increased understanding of plant and pollinator hybrid zones can influence laws and regulations regarding habitat conservation and to overall limit human impacts ecosystems. Although identifying the structure of hybrid zones accurately remains a challenge, studying them is still critical to expand our understanding of evolution and ecological biology.

Reproductive Barriers and Speciation

Reproductive barriers are factors that prevent gene flow and overall make it more challenging for hybrid species to be produced (Tonosaki et al. 2016). Two primary types of reproductive barriers, prezygotic and postzygotic, each play important roles in the formation of hybrid zones. (Coughlan & Matute 2020). Regarding pollinator and plant hybrid zone interactions, differences in flower structure or flowering time are prezygotic barriers, and incompatibility of pollen from one plant species to another is a postzygotic barrier (Tonosaki et al. 2016). These reproductive barriers serve an important role in maintaining hybrid zones. However, both are susceptible to gene flow, which can affect these barriers and ultimately skew hybrid zones (Tonosaki et al. 2016).

In order for new species to arise, reproductive barriers must evolve. It is only when two diverging populations are no longer able to interbreed and produce a viable offspring due to the evolution of prezygotic and postzygotic barriers when entirely new species are produced (Mahilkar et al. 2023). If the diverging species are still closely related, pollinators can still allow hybridization between these two plant species (See *Figure 1*). These hybrid plants often have a morphology intermediate to either parent (Cardona et al. 2020).

Pollinators and Hybrid Zones: Evolutionary Interactions

Pollinator-Driven Gene Flow in Hybrid Zones

Pollinator mediated selection are evolutionary processes that are commonly used to study reproductive isolation and how it impacts speciation. However, research in hybrid zones aims to understand the role of the pollinator itself, and how it affects the diversification of plants (Royer et al. 2020). It is believed that Joshua trees coevolved with their pollinators, *T. synthetica* and *T. antithethica*. The Tikaboo Valley is home to a hybrid zone between Joshua trees (*Y. brevifolia* and *Y. jaegeriana*) Within the hybrid zone, genotypes of 1734 Joshua trees were collected, where flowering trees were tagged with sticky traps to catch and identify the moths acting as pollinators. Measurements were made regarding the hybrid tree traits, such as floral design before DNA was extracted from the leaves. Using the genomes, a hybrid index score was assigned to each specimen to determine if the tree was *Y. brevifolia*, *Y. jaegeriana*, or a hybrid. It was determined that the role of moth pollinators were less important in shaping the hybrid zone than expected. The moths did not prefer one Joshua tree phenotype over the other, thus causing a weak phenotypic selection, low levels of fidelity (*See Figure 2)*, and showcases the complexity of hybrid zones (Royer et al. 2020).

Other pollinators constantly visit flowers of the same species, demonstrating high levels of floral fidelity. 6,713 bees across 52 species were studied across four years, concluding that 14 of the bee species show high levels of fidelity, preferring certain plant species across all 4 years (MacLeod et al. 2016). Additionally, the hawkmoth *Manduca sexta* prefers to pollinate flowers with longer limbs and shorter tubes, further driving the divergence of species (Hermann & Kuhlemeier 2011). Reduced pollen transportation of plants due to low level of floral fidelity can contribute to reproductive isolation, lower levels of gene flow, and lead to differentiation between plant species (Breitkopf et al. 2013). The shapes, sizes, or patterns of flowers can affect pollinator behavior and the likelihood that plants' genetic material is distributed. Amounts of pollinator visits for two colors of penstemon hybrid flowers, blue and fuchsia, in a hybrid zone were collected. Out of 284 total visits between bees and hummingbirds, the fuchsia morph received 74.6% of all pollinator interactions between the two, and visits lasted 4.6 seconds longer, creating opportunity for speciation of new hybrid zones (Cardona et al. 2020).

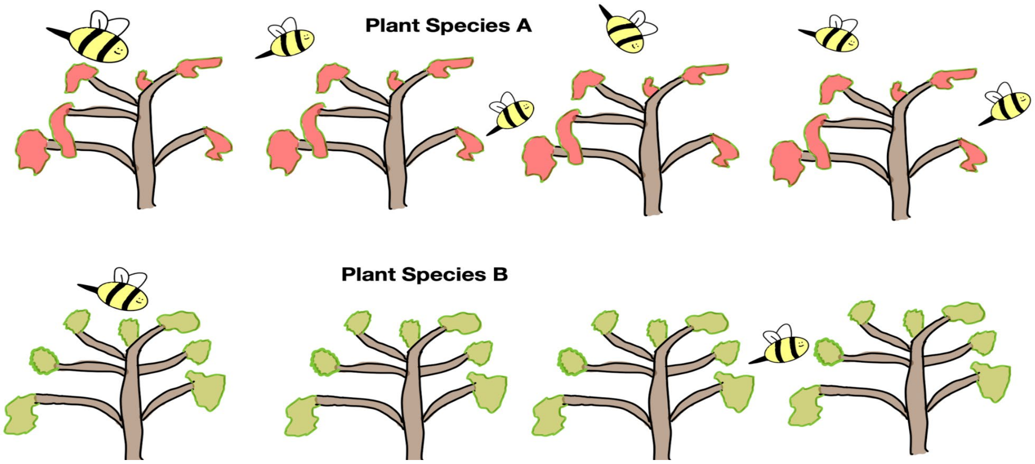

Figure 2. Pollinator Fidelity. Bees are shown visiting Plant Species A at a higher frequency than Plant Species B. Because Plant Species B is visited less often, it depicts lower levels of pollinator fidelity than Plant Species A. The consistent brown branches between Species A and Species B suggest that the difference in pollinator frequency is due to the flowers themselves, such as color, shape, or scent.

Coevolutionary Dynamics

Coevolution is when one species evolving influences a different species' evolution as well. Within a hybrid zone, coevolutionary dynamics largely influence the emergence of new species. Plant genera, such as the *Aquilegia*, have coevolved with hummingbirds with long tongues by increasing the length of their spur, changing the allele frequencies in their population (see *figure 3*). Such coevolution between plant and pollinator plays a significant role in species divergence, especially among populations of species that interact intensively (Hermann & Kuhlemeier 2011). In other regions where plants hybridize, the hybrid plants are able to produce flowers with intermediate morphologies (refer to *figure 1*). Over time, if these hybrid plants find a new pollinator that is more suited for its morphology, a feedback loop begins, further influencing speciation of both the plant and pollinator, and establishing the hybrid population (Vallin et al. 2012).

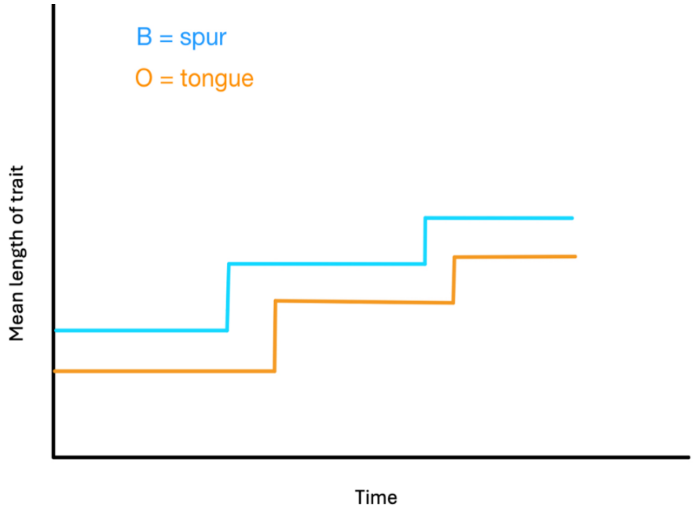

Figure 3. Coevolution of Aquilegia Flowers and Hummingbirds. Aquilegia flowers are represented in blue, while hummingbirds are represented with orange. The spur length of the Aquilegia coevolved with the tongue length of the hummingbird, promoting evolutionary change of both species.

Just as plants are able to evolve in response to a shift in pollinator behavior, pollinators can undergo similar evolution in response to changes in floral populations, densities of plant species, or climate change within hybrid zones (Gilman et al. 2012).

Recent Advancements

Due to modern technological tools, our understanding of evolution within hybrid zones between plants and pollinators has advanced within the past few decades. Historically, DNA analysis was largely limited to model organisms. However, due to the recent ability to generate molecular markers across entire genomes, a much larger range of species can be studied. Extinct species with degraded DNA can now be analyzed via exon capture, allowing scientists to gather data on species before human caused climate change (Taylor et al. 2015). It is becoming increasingly easy to gather data from multiple spots of the genome from various species and their hybrid zones. Due to an increasingly advanced set of genomic tools, new DNA sequencing technologies allow us to view real time changes in the distribution of hybrid zones, allowing scientists to monitor their location and give reason for such shifts (Taylor et al. 2015). With a combination of all these tools, scientists are better able to explain the distributions of pollinators and plants within hybrid zones that are impacted by climate change (Taylor et al. 2015).

Concluding Remarks

In this review paper, examples of introgression, reproductive barriers, and coevolution are cited to display how the spread of pollen to various plant species promotes speciation events. Pollinators are able to change the shapes of hybrid zones, and thus play important roles in plant speciation events. However, it can be difficult to pinpoint the exact cause of changing hybrid zone distributions, whether habitat loss, climate change, or chance alone (Taylor et al. 2015). Through recent advancements in DNA sequencing, it has become significantly easier to pinpoint the exact cause of hybrid zone change using distributions of pollinators and plants.

Due to recent advancements in DNA sequencing and analysis, scientists have expanded how well they understand species interactions in hybrid zones. Although these tools can improve our understanding of hybrid zones, they can also change the way we view the world. For example, through studying climate change across areas of Nepal, it's predicted that these hotter temperatures and changes in water levels will hurt the economies of poor countries (Dhital et al. 2023). It is also believed that salamander and cricket hybrid zones in the Smoky Mountains change location based on raising temperatures, affecting regional biodiversity (Chunco 2014).

Lastly, a reflection on this topic evokes an important idea: What if our understanding of species boundaries and pollinator interactions within hybrid zones is not as advanced as scientists believe? Technology will continue to advance, and better genomic and DNA sequencing tools will be created. Thus, it is very possible that scientists can change their views on species interactions, what causes speciation events, and why evolution occurs. Due to the lowering costs of genome sequencing, scientists are beginning to use environmental DNA (eDNA) to expand sample collection, estimate levels of admixture between species, and detect microorganisms within hybrid zones, something that has been historically challenging (Stewart & Taylor 2020). The one thing that is certain is that the convergence of evolution, genetics, and ecology has the potential to reshape our understanding of species interactions within hybrid zones.

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