PONTIFICIA UNIVERSIDAD CATÓLICA DEL ECUADOR

FACULTAD DE CIENCIAS EXACTAS, NATURALES Y AMBIENTALES

Ecological Significance, Development, and Occurrence of Nectar Spurs in Ecuadorian Plant Families

Monografía previa a la obtención del título de Bióloga

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Quito, 2025

CERTIFICATION

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Quito, 7 de Julio de 2025

DEDICATION

"Dedicado a mi mamá Doris, hermano Carlos, abuelitos y especialmente a mi tía abuelita, Sofía, que ha sido una fuente de inspiración para hacer las cosas de la mejor forma posible y por darme fuerza desde el cielo. A mis amigos que me han acompañado en la carrera, mi perrita Mocca que ilumina mis días y finalmente, a Alexandra Elbakyan, que hace que la ciencia sea accesible para todos."

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2. RESUMEN

Los espolones de néctar son estructuras presentes en las flores de algunas angiospermas cuya función principal es almacenar el néctar producido. Gracias a esta morfología, las flores pueden atraer a determinados polinizadores que pueden acceder al néctar e incluso “filtrarlos”. Este proceso de filtración consiste en favorecer a aquellos más efectivos para la polinización cruzada. Al obligar a los polinizadores a posicionarse de manera precisa y más cercana a la flor, los espolones facilitan que el polen se adhiera correctamente y sea transferido con mayor eficacia a otras flores. Esta estructura se ha originado y diversificado de manera independiente en múltiples clados, generando una notable variedad morfológica. Sin embargo, a pesar de su importancia ecológica y evolutiva, los espolones de néctar han sido poco estudiados, especialmente en la flora de Ecuador. La complejidad que aportan a la relación planta-visitador y su diversidad morfológica resaltan la necesidad de comprender mejor su origen, función y evolución. A nivel genético no se conocen con precisión los mecanismos o genes implicados, y la variación entre linajes, sumada a su diversificación convergente, representa un reto para la ciencia. Por esta razón, es fundamental ampliar el conocimiento sobre esta estructura, tanto desde el enfoque ecológico como genético, para esclarecer sus implicaciones. En esta revisión bibliográfica se analizó la presencia de espolones de néctar en la flora ecuatoriana, identificándose esta característica en siete familias. Este trabajo busca aportar una visión más integral sobre el papel de los espolones de néctar en la diversificación de las plantas con flores y resaltar la importancia de profundizar en su estudio, desde una perspectiva ecológica, evolutiva y genética.

Palabras clave:

Angiospermas; Convergencia evolutiva; Desarrollo floral; Espolones de néctar; Polinización.

3. ABSTRACT

Nectar spurs are structures present in the flowers of some angiosperms whose main function is to store the nectar produced. Thanks to this morphology, flowers can attract certain pollinators that can access the nectar and even “filter” them. This filtering process consists of favoring those that are more effective for cross-pollination. By forcing pollinators to position themselves more precisely and closer to the flower, the spurs facilitate proper pollen adhesion and more effective transfer to other flowers. This structure has originated and diversified independently in multiple clades, generating remarkable morphological variety. However, despite their ecological and evolutionary importance, nectar spurs have been little studied, especially in the flora of Ecuador. The complexity they add to the plant-visitor relationship and their morphological diversity highlight the need to better understand their origin, function, and evolution. At the genetic level, the mechanisms or genes involved are not precisely known, and the variation among lineages, together with their convergent diversification, represents a challenge for science. For this reason, it is essential to expand knowledge about this structure, both from an ecological and genetic perspective, to clarify its implications. In this literature review, the presence of nectar spurs in Ecuadorian flora was analyzed, identifying this feature in seven families. This work seeks to provide a more comprehensive view of the role of nectar spurs in the diversification of flowering plants and to highlight the importance of further study from an ecological, evolutionary, and genetic perspective.

Key words:

Angiosperms; Convergent evolution; Floral development; Nectar spur; Pollination.

4. INTRODUCTION

Spurs are tubular outgrowths of petals or other floral organs that typically store or produce liquid rewards, such as nectar or oil, to attract animal pollinators. These structures play a key role in the interaction between flowers and pollinators, shaping plant reproductive success, floral specialization, and potentially driving speciation events. Their presence and plasticity in phenotypes are not only adaptive traits but also important taxonomic characters in various plant lineages (Yang & Jin, 2023).

The significance of these floral features was already recognized in early botanical texts. In *Fundamenta Botanica*, Linnaeus (1736) included the presence of *nectaria*, including structures now recognized as nectar spurs, as one of the features by which corollas can differ: “Corolla differs in regard to petals, lobes, nectaries; shape, symmetry of the margin; extension; location, persistence” (Aphorism 100). Positioned within the *Fructificatio*, these traits were considered essential not only for reproduction but also for distinguishing species and understanding floral complexity. Later, he notes that if the *nectarium* is distinct from the petal, it often interacts or plays around it, denoting the variation among nectaries and listing examples such as *Aquilegia*, *Aconitum*, *Passiflora*, and *Tropaeolum*, among others (Aphorism 110). Finally, in Aphorism 125, Linnaeus points out that a nectary can make a flower with a simple structure more complex. This again highlights the early botanical attention to elaborate, potentially spur-like nectariferous structures and their taxonomic importance.

In *Philosophia Botanica*, Linnaeus (1751) expanded on his treatment of floral structures, referring to *nectaria* as the honey-producing parts peculiar to the flower (*Nectarium mel defert peculiare floris*). These were considered part of the *corolla* and used to distinguish species. He illustrated and described *nectaries* in several taxa, including horn-shaped (elongated) forms in *Aconitum* and *Tropaeolum*, which resemble what we now define as nectar spurs. This demonstrates that, even in early botanical works, structures equivalent to nectar spurs were already recognized for their reproductive and taxonomic significance.

Furthermore, In *On the Various Contrivances by Which British and Foreign Orchids Are Fertilised by Insects*, Darwin (1862) highlighted how natural selection shapes specialized traits. He went beyond merely describing this trait and sought to understand the function and ecological significance. In his observations of *Angraecum sesquipedale* he questioned the purpose of a nectary with such disproportional length, noting that only an inch and a half contained very sweet nectar. He proposed that successful fertilization of the plant depended on both the length of the nectary and the positioning of nectar exclusively at the lower and attenuated extremity. Accordingly, he hypothesized the existence of a long-proboscid pollinator, later identified as *Xanthopan morgani*, specialized in pollinating this orchid. The moth participates in the fertilization mechanism by inserting its proboscis all the way to the base of the nectary to access the nectar and subsequently withdrawing it with the pollinia attached.

Based on Darwin's observations, he described a coevolutionary race between the orchid and the moth, where longer proboscides evolved in moths to access nectar more effectively, while plants with longer spurs were better fertilized, as moths were compelled to insert their proboscides fully. He noted that such interactions could lead to the elongation of both traits over successive generations. If the nectar spur were entirely filled with nectar, it would be accessible to any moth, thereby compromising its function. Thus, nectar is located only at the base of the spur, ensuring that only a specialized moth can reach it, and leave with the pollinia attached (Darwin, 1862). Accordingly, by increasing the distance between the floral reward and reproductive structures, spurs play a crucial role in guiding pollinators and enhancing pollen transfer efficiency.

Nectars spurs have often been referred to as key innovations. Key innovations are novel traits that either open new "adaptive zones" (Simpson, 1953) or enable rapid speciation after environmental changes (Liem, 1973). These innovations are believed to drive increases in diversification, although the concept has been criticized for being difficult to test. Demonstrating how a trait impacts diversification rates can be challenging, and differences in species numbers between sister taxa can sometimes be attributed to stochastic processes (Raup et al., 1973 and Slowinski & Guyer, 1989).

Hodges & Arnold (1995) support the idea that nectar spurs are a key innovation in *Aquilegia*, showing that their evolution is linked to an increase in diversification. The study also finds that nectar spur morphology is closely related to reproductive success and isolation, suggesting a direct connection between nectar spurs and speciation. These findings provide valuable insights into understanding the mechanisms behind angiosperm diversity and the rapid speciation observed in some plant groups, addressing aspects of Darwin's "abominable mystery" (Ruse, 2020).

These specialized structures are present in approximately 3,400 extant angiosperm species (1.14% of all angiosperms), spanning at least 23 families, including Plantaginaceae, Violaceae, Ranunculaceae, and Orchidaceae, across 12 orders (S. Li et al., 2024a). The reason why so many plant lineages have independently developed nectar spurs remains unclear, as the fitness benefits of this trait are difficult to prove due to the experimental challenges involved, like they are scarce and subtle, or entirely absent in plants. Moreover, the frequency and distribution of nectar spurs within Ecuadorian flora remains unclear, leaving a gap in our understanding of how this trait may have contributed to angiosperms diversity in the region.

Nectar spur evolution appears to follow a general, though not strictly linear, trend toward elongation over evolutionary time. While reductions or losses of spurs are rare, subtle increases in length do not typically lead to speciation or a consistent trend toward ever-longer spurs. However, more substantial increases in spur length, which are sufficient to provoke shifts in pollinators, seem to drive speciation. As a result, nectar spurs can be seen as both an evolutionary dead end (difficult to shorten or lose once acquired) and as a catalyst for rapid speciation and diversification. The pollinator shift model provides an intriguing pathway in which even minor morphological changes, potentially arising from small genetic changes or single mutations, could trigger speciation events. Despite this, the precise mechanisms underlying nectar spur development and the factors influencing spur length remain poorly understood at both the morphological and genetic levels (Fisk, 2024).

5. OBJECTIVES

5.1 GENERAL OBJECTIVE

To investigate the role of nectar spurs in the pollination of angiosperms.

5.2 SPECIFIC OBJECTIVES

- 5.2.1 To investigate the ecological significance and role of nectar spur flowers in pollination.
- 5.2.2 To explore the genetic components, such as genes involved in nectar spur development and their potential functions, as well as the mechanisms behind this development.
- 5.2.3 To identify which Ecuadorian plant families exhibit nectar spur innovation for pollination.

6. THEORICAL DEVELOPMENT

6.1 NECTAR AND NECTARIES: THE ORIGIN OF NECTAR SPURS

6.1.1 NECTAR AND NECTARIES IN PLANTS

Nectar is a sweet liquid produced by plants on flowers or various parts of the plant (Lorch, 1978; Koptur, 2005). Its main function is to provide a reward to animal visitors, such as pollinators, by letting them take the liquid nourishment. The specialized parts that produce it are called nectaries which can be located inside flowers, called floral nectaries, or outside flowers, called extrafloral nectaries. The specialized glandular structures producing nectar can vary in location, such as petals, sepals, leaves, petioles or other plant parts. Depending on the position and type of nectaries, they have a specific function. Floral nectaries are involved in attracting pollinators, while extrafloral nectaries typically attract protective insects (Koptur, 2005).

6.1.2 NECTAR COMPOUNDS AND PRODUCTION

Nectar is a sugar solution mainly composed of sucrose, and it can vary on concentration (Czapek, 1922). There have been reports of some nectars rich in monosaccharides and hexose (Percival, 1961). Other compounds found in nectar besides sugars are water, amino acids, lipids, proteins, phenolic substances, alkaloids, terpenoids and even toxic compounds. It can vary from species to species the presence of these compounds and the concentration of them (Nicolson et al., 2007). Nectar can be secreted and excreted. Secretion is an active process that involves specialized glandular tissues and physiological mechanisms that synthesize and secret the nectar components. Excretion is a passive process linked to a leak or ooze out naturally without active secretion. Some hypothesis sees the excretion of nectar as a byproduct of excess carbohydrate excretion or waste removal, but there has not been experimental evidence (Koptur, 2005).

Key steps in nectar production involve activation of secretory organelles, synthesis of nectar components, secretion mechanisms and post-secretory changes and regulation. On the first step, vacuoles increase in volume, the number of dictyosomes (Golgi apparatus sacs) is reduced and endoplasmic reticulum becomes more active. Mitochondria also grow in quantity, indicative of increased metabolic activity and starch grains in the cell decline, providing energy and substrates needed in secretion. In the synthesis of nectar components, sugars are mobilized from phloem into nectariferous tissues, where they are moved through apoplastic or symplastic pathways. The production of other compounds that nectar can have like proteins and alkaloids is likely in the nectariferous tissue itself, but the sites are unknown. Although the sites are unknown, there is evidence that proteins like nectarins, are produced in the parenchyma of the nectary tissue and possess signal peptides indicating that they are secreted. Processing and vesicle formation is for proteins of nectar, where the proteins are processed within vesicles in the cells of the nectary. The secretion of nectar occurs in two main mechanisms: Holocrine and Merocrine secretion. Holocrine secretion involves programmed cell death rupture of the plasma membrane, releasing nectar contained within cells. Merocrine secretion allows secretory cells to survive and continue secreting, through vesicle fusion and exocytosis. Signal peptides for secretion participate in this activity, which is typical in to-be-exported proteins. Finally, post-secretory modification and regulation, the nectar may undergo biochemical changes, e.g., sucrose hydrolysis to glucose and fructose by invertases in the nectar. Microorganisms like yeasts will also break down nectar constituents, changing its chemistry. The plant can recycle untaken nectar, regulating secretion rates according to environmental and biotic cues (Escalante-Pérez & Heil, 2012).

6.1.3 ANATOMY AND STRUCTURE OF NECTARIES

The anatomy and structure of nectaries consists of general structural components, such as epidermis, secretory parenchymatous tissue (nectary parenchyma), sub-glandular (or non-secretory) parenchyma and vascular supply. The epidermis is the outermost tissue layer that covers the nectary. It is composed of small polyhedral cells. In the case of many floral nectaries, the epidermal cells are specialized sometimes bearing nectarostomata (specialized stomata for nectar exudation). The surface of the epidermis is covered with a thick cuticle, except where the nectar is exuded. The secretory parenchymatous tissue is located beneath the epidermis and consists of small, densely cytoplasmic, metabolically active cells. This tissue oversees synthesizing and accumulates nectar constituents, mostly sugars like sucrose. The cells have wall ingrowths which increases the surface area for transport processes, that is why they are called transfer cells. The sub-glandular (or non-secretory) parenchyma is situated beneath the nectary parenchyma. They may contain plastids that are involved in storing starch, which is converted into sugars used for nectar synthesis during secretion phases. Finally, the vascular supply, these are the vascular bundles, mostly the phloem that run close to or within the sub-glandular parenchyma. It provides for the components to nectar synthesis and its position and connectivity are crucial for efficient transport of nectar constituents (Nepi, 2007).

6.1.4 EVOLUTIONARY ORIGIN OF NECTARIES

Hydathodes have been proposed as evolutionary precursors of certain floral nectaries, specifically floral discs, due to their striking structural and functional similarities. These vegetative organs, which are primarily involved in guttation—exudation of water—are typically located on leaf margins or tips and are composed of specialized glandular tissue known as the epithem. This tissue is intimately associated with a vascular system dominated by xylem and connected with permanently open stomata or pores through which the fluid is exuded. Though primarily involved in water regulation, hydathodes sometimes yield small secretions of sugars, indicating a potential ancestral relationship to nectar secretion. The structural resemblance between hydathodes and some nectaries, notably their subepidermal glandular organization and modified stomatal features, suggests that the same basic tissue plan may have been co-opted during evolution. Genetic modularity would have allowed the re-deployment of hydathode-associated gene networks in floral contexts, resulting in the secretion of nectar rather than water. Evidence in support of such a shift is provided by nectar-secreting organs like "nectarioles" in *Chimonanthus* and *Aristolochia* with characters transitional between hydathodes and advanced floral nectaries. The hypothesis points to the evolutionary flexibility of plant structures and the way vegetative modules can be recruited to fulfill reproductive roles via developmental modifications that are quite trivial (Vogel, 1998).

6.1.5 PHENOTYPIC PLASTICITY IN NECTARIES AND NECTAR SPURS

The position of nectaries within the flower can vary, in the case of *Arabidopsis thaliana*, the nectary is located on the flower receptacle, near the base of the filaments (Smyth et al., 1990). In *Petunia hybrida*, nectaries are positioned as a ring surrounding the base of the ovary wall, with prominent development at the carpel junction (Morel et al., 2018). In Cleomaceae species, the nectaries are primarily located between the perianth and stamens (Zenchyzen et al., 2023). In Asteraceae, the nectaries form on the top of the inferior ovary and surround the style base (Frei, 1955). These examples show a clear variation of the position of a nectary in a flower, but in the case of species that possess a nectar spur, it is that they can contain the nectary within the spur or it can be placed outside the spur, serving only as a place to storage, but not for production. Examples of species that contain the nectary within the spur are *Alquilega* and *Centranthus*. In *Alquilega* the nectary is located at the base of the spur (Yant et al., 2015) and in *Centranthus* the nectar is produced by trichomes within the spur. An example of a genus that the nectary is located outside the spur is *Linaria*. In *Linaria* the nectary is positioned on the gynoecium above the spur (Cullen et al., 2023).

The floral organs from where the spur develops and number of spurs can also vary from species to species. There are flowers that contain more than one spur per flower and other just have one. *Linaria* and *Centranthus* have one spur per flower. In the case of *Alquilega*, it has one spur per petal, meaning it contains 5 nectar spurs (Cullen et al., 2023). The arrangement of the spurs, its number and how the nectar is storage bring insights into how plants can adapt to different ecological niches and how the interaction with pollinators has been carried out. This might explain the pollination strategies which, although discussed in detail in later sections, play a crucial role in the evolutionary success of the plant species.

Nectar spurs exhibit tremendous diversity in shape, length, color, and the type—or even existence—of reward they contain. While most spurs contain nectar as a reward for pollinators, others contain alternative substances such as fatty oils like in the genus *Diascia* (Scrophulariaceae) (Steiner & Whitehead, 1990), while others contain no reward at all, but have other mechanisms to attract pollinators, like some orchid species already studied by Darwin and Sprengel (Endersby, 2016). This variation not only highlights the complexity of plant–pollinator interactions but also reflects the diverse evolutionary pathways that have led to spur evolution in angiosperm lineages. The repeated, independent origin of nectar spurs in unrelated groups is a testament to their adaptive significance and suggests strong selective pressures on flower shape. This set of spur characteristics underpins both their ecological roles and their developmental complexity. To fully appreciate this adaptive significance, it is essential to examine the ecological roles nectar spurs play in shaping plant–pollinator interactions.

6.2 ECOLOGICAL ROLE OF NECTAR SPURS

The ecological role of nectar spurs has long fascinated scientists after Darwin first proposed that the length of nectar spurs might determine the availability of rewards for certain pollinators. The hypothesis generated extensive interest in the promise of nectar spurs to provide interactions between floral and faunal species. Besides pollinator selectivity, nectar spurs are now viewed as key traits in the realm of ecological specialization and evolutionary diversification. The repeated evolution seen in various plant lineages has given rise to numerous hypotheses about their function and adaptive value, notably for affecting plant–pollinator interactions and facilitating reproductive isolation.

6.2.1 NECTAR SPURS AS FILTERS FOR POLLINATORS

Nectar spurs often function as morphological filters that determine which floral visitors can access the nectar reward, consequently, pollinating the flower. *Impatiens macrovexilla* is a good example of this filter and is performed by its long, narrow spur, which conceals dilute nectar accessible only to insects with sufficiently long mouthparts, especially Lepidoptera. The floral morphology of *I. macrovexilla* aligns with the characteristics of a flower with a butterfly pollination syndrome, like pink coloration, erect dorsal petal and lack of floral scent. Some experiments were carried out to determine which, from the registered visitors, were the most effective pollinator. Diurnal hawkmoths (especially the genus *Macroglossum*) were the primary and most effective pollinators. They contributed to nearly half of all floral visits, carried *Impatiens* pollen on their proboscis and head, and were the only group responsible for fruit set, as shown in exclusion experiments. This experiment highlights the importance of field-based validation of pollination syndromes beyond assumptions based solely on floral traits (Tang et al., 2020).

In this study, the flowers were visited by other insects that were classified as pollen thieves. The reason behind this conclusion is that large bees and pollen-collecting flies, neither could reach the nectar in the spur, preventing them acting as effective pollinators. They diminished plant fitness and exerted evolutionary pressure to restrict access to true pollinators. The experiment carried out by excluding hawkmoths from reaching the flower and only leaving the large bees and pollen-collecting flies enter, showed any production of fruit. This demonstrates that none of these visitors are capable of pollinating successfully the flower (Tang et al., 2020).

However, not all *Impatiens* species with spurred nectaries exhibit this strict filtering. In *Impatiens burtonii*, the nectar spurs do not strictly filter short-proboscid pollinators. While deep nectar can be accessed by long-proboscid hoverflies later in the day, short-tongued visitors like honeybees and hoverflies pollinate effectively when nectar availability is more abundant, especially in the morning. This temporal niche partitioning suggests that spurs are partial filters and not absolute barriers (Vlašánková et al., 2017). These results counter conventional theories linking spur length to long-tongued pollinators only and uphold a broader, more versatile approach to pollination systems.

Like *I. burtonii*, *Linaria vulgaris* is a special exception to this norm. Even with its zygomorphic flower with a closed corolla and long spur, this species welcomes an impressive array of floral visitors, both long- and short-tongued bees, flies, and solitary bee species. Yet, only some of these, like *Bombus pascuorum*, are capable of successfully reaching the nectar and encountering the reproductive structures. Most other insects, like common bumblebees and wasps, indulge in nectar robbing by piercing the spur or reaching the nectar without facilitating pollination. This means that, despite the spur structure in *L. vulgaris*, there is no effort at pollination. *L. vulgaris* does not categorically exclude a broad array of visitors; however, it functions as a filter by limiting effective pollination to those visitors that possess suitable morphological attributes or behaviors (Jachuła et al., 2018). Therefore, it demonstrates that, even within ecologically generalized systems, the spur can selectively limit the visitors that are effective pollinators.

Even though spurs have been classified as structures or organs that have a filtering effect for pollinators, it does not act as a total barrier or limits to a single pollinator. What has been proved through experiments in the genus *Impatiens* and *Aquilegia* is that it only filters to one effective pollinator. For all the visitors that approach the flower, the most effective is the one that reaches the nectar located at the tip of the spur, just like Darwin suggested. It could be because of the closeness between the pollinator and the flower that allows to carry the pollen in a proper way and to take the pollen to the feminine part to be fertilized when visiting other flowers. This allows effective pollination, in contrast to the other visitors that do not have to approach the flower in that way or only steal the pollen. That is why the relation between a long-proboscid pollinator, and the length of the spur are always mentioned. Moreover, all this versatility in the visitors and how they interact with the flower generates different behaviors in the pollinators, further discussed in the next section.

6.2.2 SPURS AND POLLINATOR BEHAVIOR

Pollinator behavior can be influenced by many floral traits, and one key factor is the presence of a nectar spur. Flowering plants have evolved to develop different strategies to enhance their reproductive success, often by offering rewards to pollinators, such as nectar, to encourage them to return. When a flowering plant bears a nectar spur, it gains a specialized structure that stores these rewards in a way that can control access. By limiting which visitors can reach the nectar, spurs can influence which pollinators are more likely to return and which are most effective at pollinating. In this way, the accessibility of the reward plays a central role in shaping pollinator behavior.

Specific mechanisms developed by spurred species shape pollinator behavior. The nectar dynamics studied in two species of *Habenaria* (Orchidaceae) showed how nectar reabsorption affects the availability of nectar. Nectar secretion was followed by the reabsorption of nearly all the sugars, suggesting a mechanism to control the nectar reward over time. There was also a difference in the sugar gradient between species. In the case of this species, the nectary is located at the spur’s terminus and the sugar molecules are heavier than water molecules, so they will tend to gravitate towards the tip and rest there. The nectar sugar gradient represents two types of rewards, the pollinators with shorter proboscises will receive a more diluted nectar, with less sugar, and pollinators with longer proboscises will access the richer nectar. The difference increases with longer spurred species and this implies that a longer spur may require a deeper probing by pollinators (H. P. Zhang et al., 2023). This finding further demonstrates that the plant tends to lead the effective pollinator to the spur’s terminus to guarantee the attachment of pollinia to the pollinator.

The pressures exerted by the spurs drive pollinators to evolve longer feeding structures. In response, plants may evolve longer spurs to ensure pollinators engage closely with their reproductive structures. This pressure suggests a longer spur over time, and the shortening and vestigialization of the spur is considered unlikely (Fisk, 2024). Nevertheless, evolution and natural selection are not linear processes. In *Satyrium longicauda*, a study was conducted to explore the possibility of spur vestigialization. Elongated spurs are most likely to be effectively pollinated by a long-proboscid moth, while a short spur can be effectively pollinated by a short-proboscid insect, like a bee, making the reward accessible to a wider range of visitors. In this species, a gradual shift in pollinators occurred. The original pollinator was a moth, but occasional visits by oil-collecting bees exerted selective pressure on flowers that produced more diacetin, a compound already present in the ancestral form. This change in nectar chemistry led to a change in the morphology of the spur. Since a long-proboscid moth was no longer necessary, the spur began to shorten. This shift in pollinators altered both the chemistry and morphology of the flower. The study showed that floral traits like spurs may not evolve first but instead follow changes in pollinator behavior, particularly shifts in nectar chemistry. The morphology of the spur is not the only important trait in spurred species that can shape the behavior of pollinators. The composition of nectar also contributes to determining which pollinators visit the flower, meaning that floral chemistry and spur access work together to shape which pollinators visit and how they behave (Castañeda-Zárate et al., 2021).

Another important trait to consider beyond the structure of the spur and nectar is the rest of the floral morphology. The behavior of pollinators is also influenced by other floral parts. In *Thunia alba* (Orchidaceae), for example, large bracts play a role in the plant's reproductive success. When the bracts—structures that wrap around the pedicel and spur—were removed, the behavior of its pollinator, *Bombus breviceps*, changed. Although the frequency of visits remained the same, the way the bee accessed the reward shifted. *B. breviceps* began piercing holes in the spur to rob nectar, which reduced the plant's reproductive fitness, since this bee is the only pollinator recorded in the study area (Wu & Gao, 2024).

All the findings described in this section suggest that the behavior of the pollinator is related to many components from flower biology. It is determined from very complex characteristics and minor changes can trigger major shifts in the ecology of a species, like the pollinator shift in *Satyrium longicauda* or the pollinator behavior shift in *Thunia alba,* that comprises a wider study of non-mutualistic interactions that will be discussed in the next subsection. To have a better understanding of all this pollinator behavior, further studies should be made into more spurred species.

6.2.2.1 NON-MUTUALISTIC INTERACTIONS: ROBBERS, THIEVES AND PREDATORS

Spurs don't only attract pollinators. They also affect interactions with nectar robbers and predators. The nectar robbers represent a major challenge in reproductive fitness in spurred plants. This behavior leaves without reward to pollinators, reducing their visits to the flowers. As stated earlier, in *Thunia alba* a pollinator shifted its behavior because of the removal of its wrapping bracts, but there are insects and birds that only behave this way.

These robbers and thieves access nectar through holes in the floral structures. These organisms have adaptations to open holes, such as strong mandibles, beaks or teeth. Once the hole is created, other organisms are facilitated to also access the nectar through them. Secondary robbing can be more energy-efficient than foraging though the entry of the spur, especially for those who accessed the nectar after the hole was made (Bronstein, 2001; Lichtenberg et al., 2018; Rojas-Nossa et al., 2016).

Spurred flowers, with their commonly long and tubular floral tubes, are generally coupled with specialized pollination systems that limit access to floral rewards. These morphological characteristics can make nectar robbery less frequent because generalist visitors are unable to access the hidden nectar (Lázaro et al., 2013). The same adaptation can also enhance vulnerability to nectar robbing. When access is limited, short-tongued individuals such as some bees, honeybees, and sunbirds can enter the spur to steal nectar illegally, bypassing pollination completely (Irwin et al., 2010). In an inter-biome comparison, Rojas-Nossa et al. (2016) found that nectar robbing was more likely to occur in plants with specialized pollinator morphological adaptations, such as spurs, and particularly in wetter times when these plants were more common. Conversely, morphologically generalized flowers with open or trumpet-like shapes, broader tubes, and larger sizes were found to be more vulnerable to nectar robbery, particularly in dry seasons (Janeček et al., 2022; Klomberg et al., 2022). Therefore, whereas the spur functions to screen and guide effective pollinators, it also attracts nectar robbers when access is too limited. These findings point to a key trade-off in floral evolution: traits like spurs can defend against inefficient pollinators but, in the process, make the plant vulnerable to thieves that bypass the intended pollination route (Sakhalkar et al., 2023).

Referring to predators, there are some important implications, as the benefits of nectar spurs may diminish over time in evolutionary terms. Initially, nectar spurs encourage pollinators to approach the center of the flower, promoting efficient pollen transfer (Fisk, 2024). However, close contact with flowers can come with fitness costs for pollinators, such as increased predation risk from ambush predators like crab spiders or mantids (Morse, 1984), or the risk of carrying excess water from floral surfaces, which can hinder flight performance. These risks create pressure in pollinators opting alternative strategies, such as nectar robbing, to access nectar while minimizing these risks (Fisk, 2024).

Nectar spurs are significant in the guidance and choice of effective pollinators; however, they have a key role to play in complex non-mutualistic relationships. Morphological aspects of the spurs may deter some ineffective visitors but simultaneously invite exploitation by nectar robbers and opportunistic predators. Such processes emphasize the evolutionary compromises in the attraction of desirable partners and exclusion of those capable of reducing fitness. Understanding such interactions is essential to an overall appreciation of the ecological and evolutionary pressures shaping floral traits in spur-bearing species.

6.2.3 NECTAR SPURS AND ECOLOGICAL DIVERSIFICATION

While nectar spurs play a role in guiding pollinators and mediating interactions with exploiters, their influence extends beyond individual interactions. These structures also contribute to broader ecological and evolutionary patterns, including the diversification of species and pollination systems across different habitats.

Spurs have been characterized as good reproductive isolation since Darwin suggested this when studying orchids. The reason behind this is all the previous sections discussed before in the ecological implications of this structure. Pollinator shift that creates reproductive isolation between populations. Over time, this dynamic can drive speciation in spurred lineages, contributing to their diversification (Fisk, 2024). Spur morphology has been characterized to influence pollinators' behavior, complementing with other structures of the flower and nectar chemistry, creating a complex interaction. Studies made by Hodges (1997) stated that pollinator-mediated selection on floral traits, such as spur length, can drive reproductive isolation even in interfertile species and differences in spur traits across species are closely matched to pollinator morphologies (e.g., tongue length), reinforcing divergent evolution and pollinator-driven selection.

A well-studied example is the genus *Aquilegia*. Hodges (1997) proposed that the evolution of nectar spurs in this group represents a key innovation that enabled its rapid diversification. This conclusion is supported by the absence of spurs in closely related genera such as *Thalictrum* and *Isopyrum*, which have not diversified to the same extent. Moreover, the striking morphological variation in spur traits—such as length, orientation, curvature, and color—across *Aquilegia* species correlates with different pollination syndromes and contributes to reproductive isolation.

The role of nectar spurs in diversification is especially clear in genera like *Aquilegia*, where their interaction with pollinators has played an important role in speciation. While these patterns are seen in many plant groups, it's also necessary to understand how nectar spurs actually develop. To explore this, the next section focuses on the genetic components involved in spur formation and the mechanisms behind their development.

6.3 NECTAR SPUR DEVELOPMENT

6.3.1 ORGAN GROWTH MECHANISMS IN PLANTS

Plants induce organ development in a highly complex interplay of processes that include genetic regulation, cell division and expansion, anisotropy, tissue polarity, growth coordination, and phytohormone signaling. Genetic regulation at the root—determined by the plant's genotype—sets the potential boundaries for organ size and shape by determining the range and nature of the developmental events (Johnson & Lenhard, 2011).

Growth of organs typically begins with cell proliferation, when cells are dividing all over the whole primordium. Although the absolute increase in size is restricted currently, it correlates highly with the increase in number of cells. Cells are also increasing their cytoplasm during proliferation because of increased expression of genes that code for components of the translational machinery (Johnson & Lenhard, 2011).

One of the key development transitions is the arrest of proliferation and then shift of the plant to cell expansion. It is an essential phase, as the ultimate organ size is primarily set currently. TCP transcription factors have been shown to act as regulators of this transition, with their equilibrium between class I (growth-promoting) and class II (growth-inhibiting) TCPs determining the timing of arrest of proliferation (C. Li et al., 2005).

Cell growth ensues, largely responsible for organ enlargement as the cells grow in length. This is achieved by relaxing the cell wall matrix through the action of wall-modifying enzymes secreted and new polysaccharides deposited, extending the wall to the necessary degree to allow growth (Johnson & Lenhard, 2011).

Anisotropy, in plant biology, is the dependence of material properties or growth on directionality in plant tissues and cells. It is part of the process of plant organ development because it determines the direction of cellular growth and, in some instances, cellular division—thus playing a significant part in regulating the ultimate shape of the plant. Research information on *Zea mays* indicates that anisotropy also plays a crucial role even in specialized structures such as stomatal complexes. In this instance, the anisotropic properties of guard cell walls facilitate the reversible shape and volume changes required for normal stomatal function (Gkolemis et al., 2023).

Besides local cell behavior, polarity is also key to organ morphogenesis. It signifies coordinated orientation of cell growth across tissues and can regulate direction and form of elongation, which, for instance, is significant in specialized structures like nectar spurs. Together with polarity, growth coordination ensures that growth is properly coordinated not merely within organs and tissues, but across the entire plant body—for instance, between root and shoot (Johnson & Lenhard, 2011).

Finally, these events are tightly regulated by phytohormones. Auxin is crucial for new organ positioning and growth polarity determination, while Brassinosteroids (BR) promote cell elongation. Both hormones influence in anisotropic growth (Kim & Wang, 2010; C. Li et al., 2005). BR and the cell wall matrix are two significant parameters regulating anisotropic growth, and both of them are crucial to govern how and where the plant cell is expanding. In anisotropic root growth, BR signaling plays the most significant role in regulating anisotropy of growth. It promotes a higher anisotropy ratio of axial elongation by boosting longitudinal cell extension and limiting radial expansion. As a result, BR activity facilitates root elongation and inhibits diameter. When BR signaling is compromised, this balance is disrupted cells elongate more radially and hence roots become shorter and stouter (Khandal et al., 2025).

6.3.2 GENERAL DEVELOPMENTAL PATTERNS IN NECTAR SPURS

Nectar spurs show extreme phenotypic diversity across the various clades in which they occur, and they show considerable variation in their morphology even among closely related taxa. As with other plant organs, their ultimate morphology is the result of tightly controlled developmental processes. Interestingly, three of the most intensely investigated mechanisms have been cell proliferation, cell elongation and anisotropy, which play vital roles in specifying spur length and shape. Specifically, the influence of anisotropy, or cell directional growth, has been identified as a central mechanism in the formation of such structures, enabling the elongation of the tissues without concomitant widening.

Besides these cellular processes, more attention has been focused on the genetic foundation of spur formation. Several candidate genes have been found as potential regulators of nectar spur development, many of which are associated with more general floral or organ identity systems. Although these genetic findings are encouraging, they are also preliminary, and additional functional research must be conducted in order to validate their roles and interactions in the developmental process. Collectively, these observations point to nectar spur morphogenesis as a complex process with mechanical, cellular, and genetic aspects (S. Li et al., 2024b). The next sections will consider these processes in more detail, with an emphasis on how cellular behavior and anisotropic growth can account for the range of spur shapes.

6.3.2.1 INTERPLAY BETWEEN CELL PROLIFERATION AND ELONGATION IN SPURS

The switch between cell proliferation and elongation and how they interplay will determine organ size, like a spur. For instance, there have been many studies made in some genus bearing nectar spurs that can bring insights into the mechanisms and how they work when developing a nectar spur.

In the genus *Aquilegia*, the flowers are actinomorphic, and each petal carries a spur. There is a nectary at the tip of each spur, and it secretes nectar into the tubular structure. Early ontological studies show that the spur development in this genus is determined by a steady cell division from a meristematic zone near the base of each spur (Tepfer, 1953; Tucker & Hodges, 2005). Moreover, recent studies challenge that hypothesis and indicate that cell division occurs at an early stage throughout the nascent spur, and subsequent spur elongation is driven by anisotropic cell expansion (Puzey et al., 2012).

Subsequent studies on other genus like *Centranthus* (Caprifoliaceae), *Linaria* (Plantaginaceae), and *Epimedium* (Berberidaceae) found that each genus shows a similar pattern of spur development to *Aquilegia*: an early cell division stage, followed by a period of cell elongation. In this way, the mature spurs result in having much longer cells than nascent spurs (Cullen et al., 2018; Mack & Davis, 2015; Xie et al., 2022).

The key difference that separates the taxa studied to date is how these two stages of organ growth switch and which stage is conspicuous when determining the length of the mature spur. In *Linaria*, mature spur length is almost entirely determined by the cell division phase during early spur development (Cullen et al., 2018). In *Aquilegia*, it is almost entirely determined by the cell elongation phase (Puzey et al., 2012). *Centranthus* and *Epimedium* spurs depend on both developmental stages controlling sur length (Mack & Davis, 2015; Xie et al., 2022).

6.2.2.2. ANISOTROPIC GROWTH IN NECTAR SPURS

Anisotropic growth refers to the growth of cells in a specific direction, and it promotes spur elongation, particularly in those species in which cell elongation is more important than cell division in determining ultimate spur length. Interspecific variation in spur length in *Aquilegia* is largely regulated by anisotropic cell growth. Cells become more elongated along the longitudinal axis in longer-spurred species compared to shorter-spurred species.

This anisotropic cell expansion in fact elongates the spur without necessarily increasing cell number, demonstrating how anisotropic growth is able to fine-tune the shape of organs very precisely by altering cell form rather than cell number. In *Aquilegia rockii*, intraspecific variation in spur length can involve changes in cell number instead, suggesting some degree of flexibility, yet anisotropic cell expansion remains the dominant factor for producing large-scale variation in spur length between species (Cullen et al., 2018, 2023).

Comparative species comparisons affirm that spur-length variation almost invariably constitutes a change in a single dominant growth phase. In *Aquilegia*, for instance, 99 % of interspecific length differences are explained by alterations in cell anisotropy alone (Puzey et al., 2012; Whittall & Hodges, 2007). In contrast, *Linaria* accomplishes its spur length variation largely through differences in the number of cell divisions in the initial phase of development, with minimal alterations in cell length (Cullen et al., 2018). Surprisingly, even within one species (*Aquilegia rockii*), as mentioned before, the intraspecific variation in spur length can be entirely dictated by differences in cell number, while cell size is hardly changed (Zhou et al., 2019). Cumulatively, these investigations imply that although proliferation and elongation are both developmental "tools," each lineage tends to utilize one process over the other to produce morphological diversity. That is why examining these mechanisms across different lineages is essential to gain a comprehensive understanding of spur development and its evolutionary flexibility, an aspect that will be further discussed in the following sections.

6.3.2.3 DEVELOPMENTAL STAGES OF A SPUR IN *EPIMEDIUM*

Nectar spurs vary considerably among angiosperms, not just in their origin from different floral organs but also in form, length, direction, and coloration. Some have sepal-derived spurs—as in *Comparettia sotoana* (Orchidaceae)—and others have petal-derived spurs, like *Aquilegia* (Ranunculaceae), or a pair of petal spurs within a sepal spur, as in *Delphinium* and *Consolida* (Ranunculaceae) (Antoń & Kamińska, 2015; Pupulin & Merino, 2013). The orientation of the spurs is upward, downward, or horizontal; curvature ranges from straight to spiral; and color ranges. The existence of this morphological variation illustrates the plasticity of floral developmental pathways and indicates that although the fundamental cellular mechanisms are perhaps the same, the particular developmental strategies can be quite different in various taxa.

A particularly informative genus is *Epimedium* (Berberidaceae), which exhibits remarkable petal morphological diversity. Approximately 35 species of the genus develop spurred petals measuring from approximately 1 up to 35 mm in length, whereas other species develop saccate, shallow-pocketed, or flat petals (Yan FuLin et al., 2016; M. L. Zhang et al., 2007). The same applies for the distribution of nectary tissues: in species with three-dimensional petal shapes, nectaries are usually present at the tips of petals but lacking in species with flat petals (e.g., *Epimedium parvifolium*). This places *Epimedium* as an important system for examining the evolution of spur difference through morphological and cell differentiation.

In this genus, researchers also discovered five developmental stages of nectar spur development, based on floral bud size and petal morphology. These are: (1) primordium initiation, as petal primordia arise following sepals; (2) delayed development, a temporary halt in petal growth as stamen primordia develop; (3) petal enlargement, simultaneous with ongoing androecial development; (4) concavity formation, in which localized outgrowth is initiated based on species-specific patterns; and (5) maturation, where the concavity extends into a spur or sac-like form. These stages were identified through in-depth morphological studies and observations under scanning electron microscopy and are currently unique to the genus *Epimedium* (Xie et al., 2022). While similar phases might exist in other genera, further comparative developmental studies are needed to determine if this pattern can be extended to include all spur-bearing species. Understanding these distinct developmental phases raises a key question: what genetic mechanisms drive each stage and how conserved, or divergent, are they across spur-bearing lineages?

6.3.3 GENES CONTROLLING NECTAR SPUR DEVELOPMENT

6.3.3.3 TRAIT COMPLEXITY

Character complexity can be understood by analyzing its natural variation and how it segregates in controlled crosses. Simple traits are usually regulated by single genes, showing clear inheritance trends—dominant, recessive, or codominant. The following generations follow similar patterns and ratios, depending on the alleles of the parents. However, when F2 (second generation) shows continuous variation instead of distinct categories, it suggests polygenic inheritance, where several quantitative trait loci (QTLs) influence the trait. In these cases, environmental effects also tend to play an important role (Fisk, 2024). This strategy offers the basis for the discovery and investigation of candidate genes for complex traits, including ones that affect nectar spur morphology.

The complexity of traits like nectar spur length can be understood through variation seen in controlled crosses, but uncovering their genetic basis requires identifying the genes involved. In recent years, investigators have started trying to identify candidate genes that may regulate the development of nectar spurs, especially in model genera like *Aquilegia* and *Linaria*. Future studies using techniques like QTL mapping and comparative transcriptomics could offer deeper insights into the gene expression patterns, developmental switches, and regulatory networks behind spur diversity. The following sections outline the genetic control of spur development across different systems. To better understand which genes might be involved in this complex process, it will be important to build on findings from well-studied model and non-model systems.

6.3.3.2 INSIGHTS FROM *ARABIDOPSIS THALIANA* AS A MODEL SYSTEM

Arabidopsis thaliana as a plant model has numerous studies of how genes work to develop plant organs. Despite lack of a nectar spur, several studies have brought insights into which genes might control nectar spur development or at least play an important role.

For instance, *AUXIN RESPONSE FACTOR 3 (ARF3)* plays a role in the floral meristem regulation and organ organization patterning. *ARF3* is auxin-induced at the periphery of the shoot apical meristem, where it controls meristem–organ boundary-specific genes like *CUC1–3, BOP1–2,* and *TEC3*, thus establishing the typical pattern of organ arrangement (phyllotaxis). Interestingly, *ARF3* moves to the meristem organizing center, where it suppresses cytokinin signaling and *WUSCHEL* expression. This activity is a prime example of a pivotal function in balancing the ratio of meristem maintenance and organogenesis. The interaction between auxin and cytokinin signaling and boundary formation mechanisms must be important for the creation of new plant structures (K. Zhang et al., 2022).

Research on *Arabidopsis thaliana* has revealed that cytokinin is involved in cell division regulation during female gametophyte development through the regulation of specific cell cycle genes. Most noteworthy among them is *CYCD3-3*, which has been found to be essential, and alteration in its expression—spelled out through *CYCD3-3* mutants—results in embryo sac development aberrations. These observations point to the significance of hormonal control of cell cycle activity, for example, by the activity of genes such as *CYCD3-3*, which may play a role in the localized and oriented cell divisions that underlie spur outgrowth (J. Zhang et al., 2022). Thus, Arabidopsis provides a useful reference for the identification of candidate genes participating in the morphogenetic events of complicated floral structures in other organisms.

TCP4 transcription factor, which is regulated by miR319a, plays an essential role in the development of floral organs, while petal development is most prominently affected. Interfering with the function of miR319a leads to overexpression of the TCP genes, e.g., *TCP4*, causing constricted petals and stunted stamens. Overexpression of a miR319-insensitive form of *TCP4* causes even more extreme floral defects, including lack of petals and stamens. The results emphasize the essential function of precisely regulated *TCP4* activity in maintaining normal organogenesis (Nag et al., 2009). Given that spur development relies on the exact spatial modulation of growth in petal-derived tissues, the *TCP4*-miR319a module appears to be a versatile regulatory mechanism worth investigating in contexts where nectar spurs are developed.

Moreover, the *LONELY GUY (LOG)* gene family members in Arabidopsis thaliana are also key regulators of cytokinin activation, with several of its members activating cell division in various tissues, such as embryos and leaf vascular tissues. *LOG* genes overexpression results in phenotypes that agree with enhanced cytokinin activity, as seen through modified shoot morphology and elevated mitotic activity. In Arabidopsis, the capacity of *LOG* genes to regulate cytokinin activity and induce local growth points to their involvement in developmental processes involving proliferation at the cellular level (Kuroha et al., 2009). Thus, *LOG* genes emerge as promising candidates for investigation in cases where spur development relies on tightly regulated hormone-induced cell division.

These findings demonstrate how *Arabidopsis* studies, despite the lack of nectar spurs, provide important molecular insights that contribute to the discovery and clarification of candidate genes potentially associated with spur initiation and development in non-model systems.

6.3.3.3 SPUR INITIATION

Genetic control of spur development has been studied in *Linaria vulgaris* through a transcriptomic analysis. Cullen (2019) and Cullen et al. (2023) compared a spurred species (*L. vulgaris*) and a non-spurred species (*Antirrhinum majus*). *A. majus* contain a structure like a spur called gibba, and it has been referred as the precursor of a spur. It is more a pouch-like structure, less conspicuous than a spur but contains nectar. They established different developmental points when they evaluated the flowers: eight to nine days prior anthesis, four to five days prior anthesis, and two days prior anthesis. These timepoints were established to understand the genes involved in the initiation process of a nectar spur, but not the genes involved in the onset of spur development. Another important factor in this research is that they studied gene expressions on different parts of the flower: ventral petals (the ones bearing the spur and gibba) and dorsal petals (which do not).

The researchers found that the genes involved in spur initiation are likely to be differentially expressed between ventral and dorsal petals of *L. vulgaris.* In the case of *A. majus*, their orthologues are less differentially, highly or simply not expressed. Genes involved in the initiation of spur development were expected to decrease their expression over time, while genes controlling spur maturation were expected to increase and show peak expression in middle or later timepoints and they were classified as “spur aid” genes.

Spur aid genes were found to control cell division or cell elongation (important stages of the development of an organ) and many of them were orthologues of genes known to control such functions in *Arabidopsis*. Some of the *Linaria* orthologues like *POPOVICH* (POP)*, ARF* family and *TCP8*, were identified as spur aid genes. *POP, ARF6, ARF8,* and *TCP4* are thought to contribute to spur development in *Aquilegia*, indicating that the distantly related clades *Aquilegia* and Antirrhineae may have independently recruited similar genes or genetic pathways to drive nectar spur evolution (Cullen, 2019; Cullen et al., 2023).

6.3.3.4 GENETIC CONTROL OF NECTAR SPUR DEVELOPMENT IN *AQUILEGIA*

Most studies have been conducted on this genus, due to the lack of a spurred model system and the variation among these species. Due to the different elongation patterns in this genus researchers have focused on investigating the genes behind spur elongation comparing the expression of genes between long and short spurred species. Previous studies have shown that the spur length can be predominantly attributed to variation in cell elongation.

According to the study of R. Zhang et al. (2020), the genes *ARF6* and *ARF8* are involved in petal spur development, silencing these genes in *Aquilegia coerulea* leads to significantly shorter petal spurs, primarily due to the reduction of anisotropic growth, especially near the nectary region. This suggests that spurs are sensitive to the loss of ARF6/8. Another finding in this research are defects in the maturation of nectaries, which prevents nectar secretion and is associated with reduced expression of *STY1* and *STY2*. These findings indicate that *ARF6* and *ARF8* promote spur elongation by enhancing cell expansion and contribute to the proper development of functional nectaries.

STY genes have a fundamental role in the development of floral nectary within the petal spur. A study made by Min et al. (2019) made an identification through RNAseq analysis, followed by phylogenetic and expression studies, and functional knock-down experiments. In early stages of flower development, the expression of *STY1* shifts from the distal petal tips to the base of the developing spur, where the nectary usually forms. The silence of *STY* homologs (*STY1*, *STY2* and *LRP*) disrupted nectary development and nectar production, confirming the role of these genes in this process. Another important finding in this study is that these genes are not auxin dependent. They tested their relationship by adding auxin, indole-3-acetic acid (IAA), and it did not induce nectary development, and known *STY* targets involved in auxin biosynthesis where not expressed in the spur. This suggests that *STY* genes in *Aquilegia* determine nectary identity through a mechanism distinct from their traditional role in auxin regulation.

Kramer et al. (2007) silenced a gene, homologue of the B-class floral homeotic gene *PISTILLATA* (*PI*) in *Aquilegia* using a virus-induced gene silencing (VIGS). The experiment showed that the flowers with strongly silenced *PI* had a reduced or absent spur (the ones that originate from a petal), suggesting that this floral identity genes could control the petal-derived spur.

TCP4 is important in the control of cell division, particularly in the distal region of the nectar spur of *Aquilegia coerulea*. Downregulation of *TCP4* expression in the plant resulted in heightened proliferation of cells in only the distal compartment and not in the proximal compartment or the petal blade. This is an indication that the development of the spur is modular, and regions are controlled independently (Yant et al., 2015).

Remarkably, *TCP4*-silenced spurs also displayed changed cell wall orientation, suggesting that mechanical tension during early development can impact cell behavior, possibly modulating auxin distribution and cytoskeletal alignment. These observations suggest that *TCP4* suppresses cell divisions in specific regions of the spur, defining its final shape and permitting the pattern of elongation typical of *Aquilegia* spurs. Its function was revealed by *TCP4* gene silencing experiments that also serendipitously revealed its spatially restricted function to control proliferation during spur formation (Yant et al., 2015).

A study made in *Aquilegia* species with a variation of long and short spurs was made to compare the expression of genes. It was determined that the difference in length is mainly a result of changes in cell number. Short spurred species showed a down-regulation of genes associated with cell division (*F-box* gene with an NHL repeat domain, *CDKB2-2*, and *LST8*). This finding suggests that these genes control spur length. Moreover, they concluded that the principal difference between spur length is made by cell division genes and not hormone-related genes (Zhou et al., 2019).

Other studies made in *Aquilegia* show that the gene *POPOVICH* (*POP*) has been studied and determined that is necessary for both spur and nectary development. It promotes cell division in the spur cup during early development and the loss of it could lead to spur loss or shortening and absence of nectaries. Also, the gene *JAG*, that promotes cell proliferation in lateral organs, including petals, is important. It is not spur specific, but the knockdown of it results in loss of spur and nectary, suggesting that is necessary for spur development (Ballerini et al., 2020).

Together, *Aquilegia* studies have uncovered a complex genetic framework underpinning nectar spur development. This machinery comprises both general regulators of organ identity and growth and those specific to spur development. The dominant factors *ARF6, ARF8, STY1/2, PI, TCP4, POP,* and *JAG* display the sophisticated interplay of cell proliferation, expansion, and regional specification required to generate the characteristic petal spur. These observations offer a useful context for interpreting spur development and pave the way for examining whether the same or different mechanisms are involved in the formation of spurs in other genera, like *Tropaeolum*.

6.3.3.5 GENETIC CONTROL OF NECTAR SPUR DEVELOPMENT IN *TROPAEOLUM*

Unlike *Aquilegia*, where spur elongation has been well characterized, the genus *Tropaeolum* remains less studied but shows promising markers like *HIS4* and *TCP4L2*. A study was conducted based on different general developmental stages established for a flower in this study. The gene *HIS4*, a marker of cell division, at stage 5, when the spur initiates, becomes concentrated in the adaxial region of the floral tube. At stage 6 and 7, the spur elongates, and the expression persists in the region. By stage 8, there is a change in the expression to the inner layers of the spur, where the nectariferous tissue develops. The dynamic in the expression of this gene suggests that it is important for spur growth and nectary formation. In this genus the gene *TCP4L1* and *TCP4L2* are highly expressed in the epidermis of reproductive apices, flower meristems and flower organ primordia. *TCP4L1* is continuously expressed in the inner and outer epidermis and *TCP4L2* is more specific for the nectariferous tissue (Martínez-Salazar et al., 2023).

6.3.3.6 GENETIC CONTROL OF NECTAR SPUR DEVELOPMENT IN *LINARIA*

Not many studies have been conducted in the genus *Linaria* but have been proposed to be a good candidate for a spurred model by Fisk (2024). Also, he conducted several studies to demonstrate the role of genes that were previously candidates for spur development.

CYCD3-3 is the only candidate gene that was currently implicated in controlling the early cell division stage. This gene also may be important in controlling the ventral-specific development of the spur. *ARF3* expression increased throughout spur development, suggesting that it plays a role in spur formation (Fisk, 2024). *TCP8* was identified as a gene potentially involved in spur formation in *L. vulgaris* through transcriptomic analysis (Cullen et al., 2023).

LSH3 shows a high differential expression between the ventral and dorsal petals at an early stage of floral development in *L. vulgaris,* and the expression decreases when the spur is mature. *ICR2* is differentially expressed in the cell elongation phase, suggesting that it may play a role in the elongation of the nectar spur. *LOG1*, which activates cytokinin, has been more expressed in the ventral petal than the dorsal petal throughout early development in *L. vulgaris*, but in *L. clementei* than in *L. becerrae* was less highly expressed, suggesting that spur length depends on cytokinin levels (Cullen et al., 2023; Fisk, 2024).

POP has been shown as a poor candidate gene for spur development in *Linaria*, since it is expressed at similar levels in the dorsal and ventral petals in long and short spurred species (*L. becerrae* and *L. clementei* respectively). *KNOX* genes, *HIRZ* and *INA* were known to be adequate candidates, but a study had little evidence to support or reject this hypothesis.

HIRZ was more highly expressed in the ventral petal than in dorsal petals at developmental stages in *L. becerrae* and *L. clementei*, indicating a possible function in spurred ventral petal development. Moreover, *HIRZ* is situated near the big region of interest on chromosome three delimited by Bulked Segregant Analysis (BSA), which has implications for possible genetic linkage with nectar spur determination genes (Fisk, 2024).

Together, these Linaria studies underscore how spur development may be controlled by a diverse set of lineage-specific and conserved genes. To place these candidates in context, and to highlight overlaps with other spurred lineages, the next section compiles the most frequently implicated genes across plant systems.

6.3.3.7 A SUMMARY OF IMPORTANT GENES FOR NECTAR SPUR DEVELOPMENT

The main genes involved in nectar spur development are summarized in Table 1. This table compiles key regulatory and structural genes identified across various model species, highlighting their roles in spur initiation, growth, and morphological diversification. The summary provides a comparative overview to support further studies on the genetic basis of spur formation.

Having explored the genetic foundations behind nectar spur development, it is equally important to examine the diversity of these structures across plant groups, particularly within Ecuador's rich and unique flora. The following sections address the general diversity of nectar spurs and provide a focused overview of Ecuadorian families bearing this characteristic, setting a foundation for ecological and evolutionary studies in the region.

6.4 DIVERSITY OF NECTAR SPURS IN ECUADORIAN FAMILIES

Having explored the genetic foundations behind nectar spur development, it is important to also consider the diversity of these fascinating structures across different plant groups. Ecuador, renowned for its rich biodiversity, harbors a wide variety of angiosperm families exhibiting nectar spurs. The following section provides an overview of nectar spur diversity within Ecuador's unique flora.

6.4.1 GENERAL DIVERSITY

Nectar spurs display significant variation in their shape, color, orientation, and function, contributing greatly to the diversity of floral forms (Hodges & Arnold, 1995 and Hodges, 1997). Nectar spurs can be found in a variety of plant orders, including Gentianales, Lamiales, Dipsacales, Asterales, Ericales, Brassicales, Myrtales, Malpighiales, Fabales, Ranunculales, Liliales, and Asparagales. Among these, Ranunculales is considered the most basal order, representing one of the earliest groups where nectar spurs likely evolved. This diversity across plant orders highlights the widespread occurrence of nectar spurs (S. Li et al., 2024a).

6.4.2 ECUADORIAN FAMILIES BEARING NECTAR SPURS

Families corresponding to the orders listed before were revised in The Flora of Ecuador to provide a brief description of the nectar spur. Nectar spurs were found in only seven families: Balsaminaceae, Gentianaceae, Lentibulariaceae, Orquidaceae, Papaveraceae, Plantaginaceae and Violaceae (see Figure 1 for an example of the nectar spur from one species within each family). Over the years, several taxonomic revisions have led to the reclassification of some spurred species; for example, species formerly placed in Scrophulariaceae are now classified within Plantaginaceae. However, the spur descriptions for these species are based on the Flora of Ecuador treatment under Scrophulariaceae. For certain families such as Balsaminaceae and Violaceae, an official Flora of Ecuador treatment is not yet available, but these families are well documented in the literature as possessing nectar spurs. The Table 2 highlights the diversity of spur morphology among families present in Ecuador.

Understanding these variations is essential for future studies on plant–pollinator interactions, ecological specialization, and the evolutionary history of spurred groups in Ecuador. By recognizing both the morphological diversity and the current taxonomic gaps, this work sets the stage for broader research and conservation efforts focused on these unique floral traits.

7. CONCLUSIONS

1. Nectar spurs constitute far more than ornamental features in plant morphology. They play a dynamic role in mediating reproductive success, shaping pollinator specialization, and influencing broader evolutionary trajectories.
2. Beyond simply offering a reward, nectar spurs actively shape pollinator behavior by filtering which visitors can access the nectar and effectively transfer pollen. The structure of the spur influences how a pollinator approaches the flower, and in doing so, reinforces specific pollination pathways. This ability to control access makes the spur an ecological gatekeeper and highlights its importance in plant–pollinator coevolution.
3. Longer spurs tend to favor pollinators with longer feeding structures, driving reciprocal adaptation. While this tight relationship can encourage specialization and diversification, it also comes with fitness costs, such as increased vulnerability to predators. Despite this, spur elongation remains a consistent trend, likely due to the reproductive advantages of precise pollination.
4. Changes in spur morphology—length, curvature, color, or nectar chemistry—can trigger shifts in pollinator assemblages. These pollinator shifts have ecological consequences and may contribute to reproductive isolation and speciation. However, these same traits can also attract nectar robbers or thieves, which access floral rewards without providing pollination services.
5. Genetically, the development of spurs remains a topic in need of further study. Genes identified in Aquilegia—such as ARF6/8, STY1/2, TCP4, JAG, and CDKB2-2—appear to play roles in spur elongation, cell division, and tissue identity. However, since spurs have evolved independently in multiple lineages, it's likely that different developmental pathways exist—a hallmark of convergent evolution. This makes it difficult to generalize findings from a single genus.

6. Worldwide, nectar spurs are reported in at least 23 plant families; however, in Ecuador they are found only in seven families: Orquidaceae, Balsaminaceae, Gentianaceae, Lentibulariaceae, Plantaginaceae, Violaceae, Papaveraceae. This reduced number of spurred families does not match the overall floral diversity of Ecuador. One possible explanation is that the Flora of Ecuador is outdated and incomplete for certain families, leaving a significant gap in current knowledge.
7. Despite the advances made so far, important knowledge gaps remain. The genetic regulation of spur development is still incomplete, especially across different families. The interaction between floral traits and pollinator communities also needs further field-based studies, particularly in underexplored regions like the Andes or the Amazon. Integrating ecological data with molecular and developmental approaches could lead to a more holistic understanding of this trait.

   Understanding nectar spurs holds practical potential. By shedding light on the intricacies of plant–pollinator interactions, this knowledge can be applied to conservation biology, helping to protect vulnerable species with highly specialized pollination systems. In agriculture, insights from spur biology could guide crop breeding strategies aimed at enhancing pollination efficiency. Far from being a niche topic, the study of nectar spurs reveals the deep interdependence between plants and their pollinators—one that continues to shape biodiversity today.

8. RECOMMENDATIONS

Although nectar spurs have been studied from diverse perspectives, many areas still require further exploration. The ecological aspects of nectar spurs have been clearer since Darwin's time, but new findings about the complex dynamics between visitors and plants show that further studies need to be done in this area. The spur's function is not only to attract pollinators but also to filter them, modify their behavior, and expose the flower to nectar robbers. Field research will help document these interactions, especially in biodiverse ecosystems like Ecuador, where spurred forms may have unique interactions yet to be documented.

In addition to these ecological gaps, another major gap in knowledge is in the genetics of nectar spurs. The mechanisms behind them are clearer in some genera, but this doesn't provide a complete understanding of how a nectar spur develops and evolves in different plants. For example, as vital as the work done with *Aquilegia* has been in providing genetic insights, it does not capture all evolutionary paths linked with this character. To understand differences between lineages, in *Aquilegia* the gene *POP* is vital for spur development, but in *Linaria* it appears to have little relevance. The suggestion to use *Linaria* as a spurred model, as put forward by Fisk (2024), may prove to be a significant advance, but one that still needs to be complemented by research on other clades to get a more complete picture of the range of developmental mechanisms involved.

Beyond genetic aspects, understanding local diversity is also limited because, the *Flora of Ecuador* and other books that record Ecuadorian plants need to be updated. The lack of recent and complete information limits how well we can know the real diversity of nectar spurs in the country. Without updated taxonomic work and detailed descriptions, many species with spurs might be missing or described incorrectly. This problem doesn't just affect nectar spur studies; it impacts many other botanical investigations too. Updating these resources is necessary not only to improve knowledge about nectar spurs but also to support many other ecological and conservation studies in Ecuador.

Finally, it’s important to highlight why this research matters, it is important not only for understanding plant evolution but also for protecting and maintaining ecological balance. Knowledge of how nectar spurs attract specific and effective pollinators can be applied in agriculture and conservation efforts. It can help develop strategies for improving pollination in endangered wild plants and certain crop types, thus supporting biodiversity conservation. In this way, nectar spur research has profound implications for ecological balance and conservation biology.

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10. FIGURES

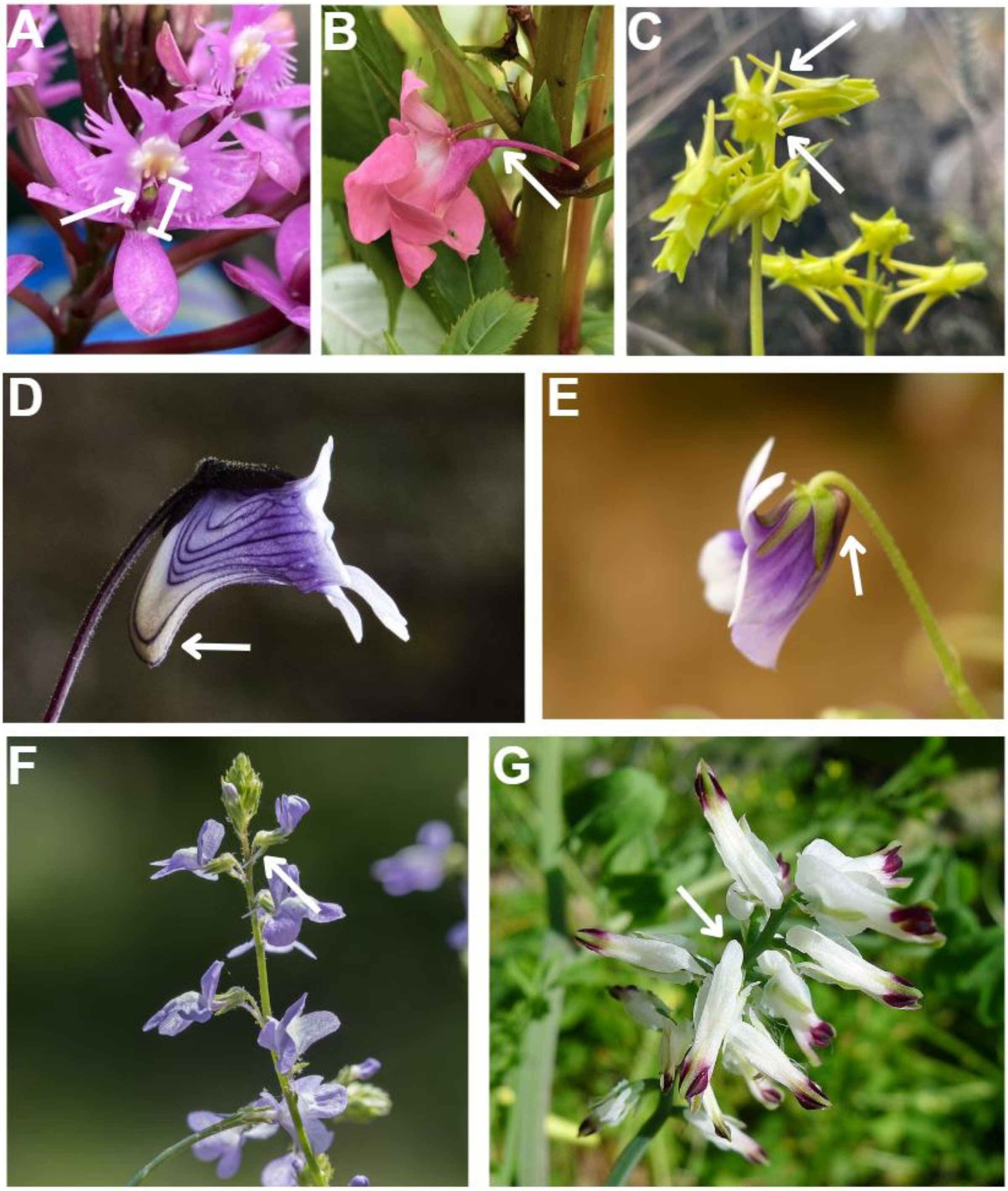

Figure 1. Examples of species with nectar spurs from plant families known to bear this structure in Ecuador. All arrows indicate the location of the spur on each flower. In some cases, the spur appears pouch- or cup-like due to its thicker and shorter form, rather than being elongated and narrow. However, all spurs are positioned at the base of the flower. **A.** *Epidendrum jamiesonis* (Orchidaceae). The double-headed arrow indicates the length of the basal spur. Photo by: dali4 (iNaturalist). **B.** *Impatiens balsamina* (Balsaminaceae), a cultivated species in Ecuador, with a well-developed and conspicuous curved spur. Photo by: miaunt (iNaturalist). **C.** *Halenia weddelliana* (Gentianaceae). Each flower contains four long, narrow

spurs. Photo by: eric_haavind_berman (iNaturalist). **D.** *Pinguicula jimburensis* (Lentibulariaceae), a carnivorous species with a thick, cup-like spur extending from the flower base. Photo by: kabirbosques (iNaturalist). **E.** *Viola scandens* (Violaceae). The spur is pouch-like and located at the back of the flower. Photo by: arjonker (iNaturalist). **F.** *Linaria canadensis* (Plantaginaceae), a cultivated species in Ecuador. It displays elongated, tapering spurs. Photo by: tlit46 (iNaturalist). **G.** *Fumaria parviflora* (Papaveraceae). The spur appears as a small, sac-like projection on the perianth. Photo by: gailhampshire (Flickr, CC BY 2.0).

11. TABLES

Table 1. Summary of key genes involved in nectar spur development across different genera. The table includes genes commonly implicated in spur initiation, elongation, and nectary formation. This summary serves as a comparative reference for identifying conserved and divergent genetic mechanisms across *Aquilegia*, *Tropaeolum*, and *Linaria.*

Gene	**Species Studied**	**Main Function in Spur Development**
ARF6 / ARF8	*Aquilegia*, *Linaria*	Promote cell elongation and anisotropic growth in spurs; also necessary for nectary maturation.
STY1 / STY2 / LRP	*Aquilegia*	Control nectary identity and development; function independent of auxin; localized to the base of developing spurs.
PI (PISTILLATA)	*Aquilegia*	B-class floral identity gene; its silencing causes loss or reduction of petal-derived spur.
TCP4 / TCP8 / TCP4L1 / TCP4L2	*Aquilegia*, *Linaria*, *Tropaeolum*	Regulate cell division patterns; particularly TCP4 restricts division in distal regions for spur shaping. TCP4L2 is associated with nectariferous tissue in *Tropaeolum*.
POP (POPOVICH)	*Aquilegia*	Essential for early cell division in spur primordia and nectary development. Poorly expressed or uncorrelated with spur development in *Linaria*.
JAG	*Aquilegia*	Regulates cell proliferation in lateral floral organs; its absence causes loss of spur and nectary.
CYCD3-3	*Linaria*	Controls early cell division specific to ventral petal; proposed to initiate spur development.

ARF3	*Linaria*	Expression increases during development; likely involved in overall spur formation.
HIRZ	*Linaria*	Shows ventral-specific expression; located near QTL for spur length; may be linked to ventral petal outgrowth.
ICR2	*Linaria*	Expressed during cell elongation phase; potentially contributes to spur length.
LOG1	*Linaria*	Involved in cytokinin activation; expression correlates with spur length variation across species.
LSH3	*Linaria*	Differentially expressed early in ventral petal; downregulated at maturity; suggests role in early spur initiation.
CDKB2-2* / F-box (NHL) / *LST8	*Aquilegia*	Downregulated in short-spurred species; involved in cell division, contributing to spur length differences.
HIS4 (TIHIS4)	*Tropaeolum*	Marker for cell division; expression tracks spur initiation, elongation, and nectary tissue formation during floral development.

Table 2. Plant families found in Ecuador that possess nectar spurs, summarizing the variation in spur morphology including shape, length, and floral position.

Order	Family	Brief description of the spur
Asparagales	Orchidaceae	Nectar spurs are tubular or curved extensions, typically arising from the labellum or sepals. Their length, shape, and curvature vary widely among species, often forming slender tubes or sacs that contain nectar. The subfamilies Orchidideae and Epidendroideae have spurred species (Garay, 1978).
Ericales	Balsaminaceae	An introduced or cultivated family in Ecuador. The spur is curved and rarely spur absent (Xu & Deng, 2017).
Gentianales	Gentianaceae	In *Halenia*, nectar spurs are elongate extensions of the corolla tube, though they can range from small protuberances to well-developed spurs. These structures may be reduced or absent in lower or later flowers of typically spurred species (Pringle, 1995).
Lamiales	Lentibulariaceae	The spur in this family is highly variable in length, ranging from long to very short structures (Taylor, 1975).
Lamiales	Plantaginaceae	The corolla tube is often ventrally spurred or saccate. Mostly introduced or cultivated plants, tghis floral trait is observed in genera such as *Antirrhinum*, *Linaria*, *Misopates*, *Cymbalaria*, *Maurandya*, *Angelonia*, and *Galvezia* (Holmgren & Molau, 1984).
Malpighiales	Violaceae	Nectar spurs are typically formed by the lower petal and vary in length from short to elongate, depending on the species (Wahlert et al., 2022).
Ranunculales	Papaveraceae	In Ecuador, the only representative of Papaveraceae with a floral spur is the genus *Fumaria*. In this genus, the spur is formed by the upper outer petal, which bears a tubular projection into which a nectary, derived from the upper stamen, extends (Lidén, 1995).