

The genetic control of herkogamy

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ABSTRACT

Herkogamy is the spatial separation of anthers and stigmas within complete flowers, and is a key floral trait that promotes outcrossing in many angiosperms. The degree of separation between pollen-producing anthers and receptive stigmas has been shown to influence rates of self-pollination amongst plants, with a reduction in herkogamy increasing rates of successful selfing in self-compatible species. Self-pollination is becoming a critical issue in horticultural crops grown in environments where biotic pollinators are limited, absent, or difficult to utilise. In these cases, poor pollination results in reduced yield and misshapen fruit. Whilst there is a growing body of work elucidating the genetic basis of floral organ development, the genetic and environmental control points regulating herkogamy are poorly understood. A better understanding of the developmental and regulatory pathways involved in establishing varying degrees of herkogamy is needed to provide insights into the production of flowers more adept at selfing to produce consistent, high-quality fruit. This review presents our current understanding of herkogamy from a genetics and hormonal perspective.

Keywords: floral development, herkogamy, mating systems, phytohormones, pollination, stamen development, style development.

Overview

Herkogamy (from the Greek for fence – *herko* and marriage – *gamos*) is broadly defined as the spatial separation of anthers and stigmas within complete flowers. It is a key composite floral trait that introduces variation in plant mating systems and is central to promoting outcrossing as well as reducing interference between male and female functions (Webb and Lloyd 1986). As a functional trait, herkogamy has long been considered an important component of reproductive interactions in angiosperms and plays a significant role in outcrossing rates and autofertility. Floral structures define herkogamy, with the spatial positioning of the ‘female’ receptive stigma and ‘male’ pollen producing anthers separated either vertically, laterally or both. The height of the stigma depends on the shape and size of the gynoecium (female organ comprising the ovary, style and stigma). The position of the anthers is determined by the length and angle of the filament that supports the anther.

Herkogamy types were described in detail in a seminal review by Webb and Lloyd (1986) and have been grouped into several additional categories, summarised in Table 1. The three main classes of herkogamy are: (1) homomorphic herkogamy; (2) reciprocal herkogamy; and (3) interfloral herkogamy. Homomorphic herkogamy is found in populations where all of the flowers have a similar morphology. Within homomorphic herkogamy, flowers can be ordered or unordered. In unordered herkogamy, there is still physical separation of the anthers and stigma but the spatial separation between them may be so small that pollinator movement between reproductive organs is disorganised or haphazard (i.e. pollinators repeatedly alternate between organs). In ordered herkogamy, there is a distinct physical separation of anthers and stigmas facilitating the likelihood that pollinators encounter one organ before the other when entering or exiting the flower. Ordered types include approach herkogamy (stigmas protruding beyond the anthers so that pollinators are likely to contact stigmas first) and reverse herkogamy (stigmas positioned behind or below the anthers so that pollinators contact anthers first) (Fig. 1).

Table 1. Hierarchical herkogamy classes.

Type	Class	Order	Description
Homomorphic	Unordered		All floral morphs of one form; complete flowers
			Pollinators contact anthers and stigmas within a flower in no ordered sequence
	Ordered		Typically, pollinators make contact with pollen and stigmas in an ordered fashion
		Approach	Stigmas extend forward of the anthers so that they contact pollinators first
		Reverse	Anthers presented forward of the stigma so that pollinators may contact them first
		Movement	Stigmas and/or anthers moved into or out of presentation position
		Lateral	Style presented horizontally on an angle away from the centre of flower
Reciprocal		Two or more floral morphs of complete/perfect flowers but differing in position of presentation of anthers or stigmas. Heterostyly, enantiomorphism	
Interfloral		Two or more different floral morphs, with anthers and stigmas presented either in part or in whole within separate morphs. Monoecism, gynomonocism, andromonoecism, dioecy	

Modified from Webb and Lloyd (1986).

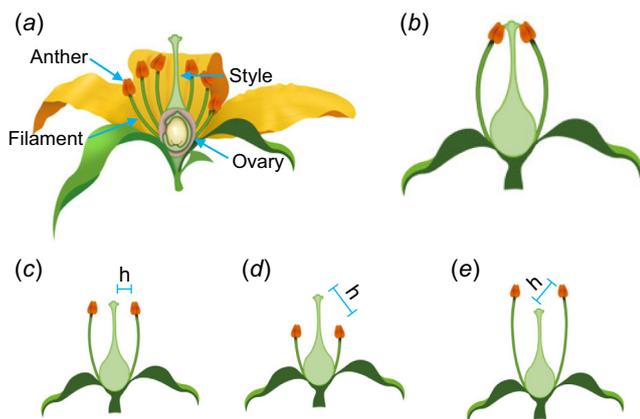


Fig. 1. Floral structure and homomorphic types of herkogamy. (a) Flower structure showing four whorls of organs – sepals, petals, stamens and carpels. (b) Hypothetical flower with petals removed showing no herkogamy. (c) Lateral herkogamy. (d) Approach herkogamy. (e) Reverse herkogamy. ‘h’ represents the herkogamy distance.

Movement herkogamy occurs in floral morphs where either the stigma or anthers move relative to each other, changing the separation as the flower develops or responds to pollination signals. Situations where anthers and stigmas are in direct line with each other may be referred to as neutral herkogamy, whilst no (or zero) herkogamy would imply the organs being in direct contact. Lateral herkogamy, where styles are horizontally positioned on an angle away from the centre of the flower, is the least common but can be seen in *Linum* (Ruiz-Martín *et al.* 2018), *Centaureum* (Brys and Jacquemyn 2012) and *Hylocereus undatus* (Cho and Ding 2021). Some species, such as *Lysimachia arvensis* exhibit both lateral and vertical herkogamy (Jiménez-López *et al.* 2019).

Reciprocal herkogamy is classed as either heterostyly or enantiostyly/enantiomorphy (Webb and Lloyd 1986; Almeida *et al.* 2013, 2018). Heterostyly (Fig. 2) refers to

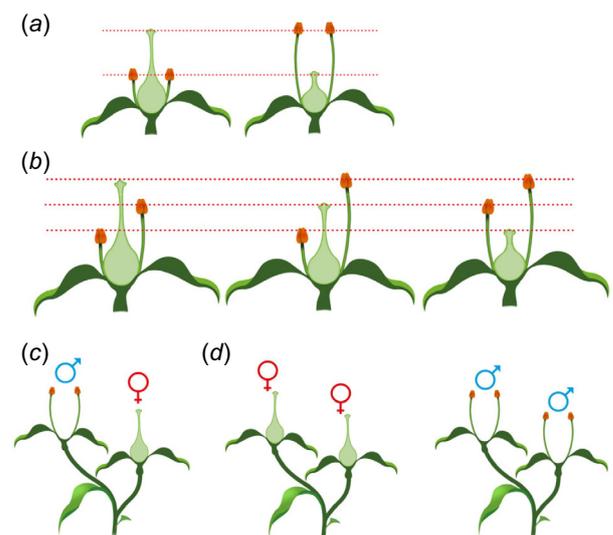


Fig. 2. Variations in floral structures. (a) Distyly. (b) Tristyly (anther and stamen lengths displaying enantiomorphy). (c) Interfloral herkogamy: monoecism (two floral morphs within a plant). (d) Dioecy (plants have either only ‘male’ or ‘female’ flowers). Dotted red lines indicate pollen transfer between organs of different morphs.

the system where herkogamy is vertical and presented reciprocally in two (distyly) or three (tristyly) floral morphs (Arunkumar *et al.* 2017). Effectively, distylous species have two different floral morphs (short-styled S-morphs, or thrums; and long-styled L-morphs, or pins) with one presenting approach herkogamy and the other reverse herkogamy. In tristylous species, a third floral morph is present that has an intermediate herkogamy profile relative to the other two morphs. In some reproductive systems, the flowers of a particular morph may only be compatible with those from different morphs – demonstrating organ separation through space working in concert with incompatibility systems (Barrett and Harder 2005; Arunkumar *et al.* 2017).

Interfloral herkogamy is when a plant contains two or more different floral morphologies, with anthers and stigmas presented either in part or in whole within separate morphs and includes monoecism (Fig. 2) (separate male and female flowers on an individual plant), gynodioecism (female and complete flowers on the same individual plant) and androdioecism (male and complete flowers on individual plants of a species).

Homomorphic, ordered, approach herkogamy is the most common amongst self-compatible species and tends to be associated with a limited number of pollinator species or functional groups (Opedal 2018). It can be found in the families Liliaceae, Amaryllidaceae, Boraginaceae, Convolvulaceae, Caprifoliaceae, Ericaceae, Papaveraceae and Gentianaceae (Kerner and Oliver 1902). The relationship between diverse pollinator communities, functional groups and plant reproductive success will not be covered in detail here but has been treated extensively by others (Fontaine *et al.* 2006; Albrecht *et al.* 2012; Ollerton 2017).

Function and evolution of herkogamy

Herkogamy has long been considered an important component of reproductive interactions in angiosperms and has been demonstrated to play a significant role in outcrossing rates and autofertility. While traditional interpretations of herkogamy posit its role mainly as a promoter of outcrossing (thus a mechanism to reduce selfing), its presence in species that are genetically self-incompatible, as well as the presence of other promoters of outcrossing such as dichogamy and dioecy, suggests it may also serve to prevent or reduce anther-stigma self-interference within a flower (Galen and Gregory 1989; Waser and Price 1991; Fetscher 2001). Self-interference may occur when self-sourced pollen saturates the stigmatic surface and clogs it so that outcrossed pollen is restricted from making contact and germinating, or when stamens remove pollen from stigmatic surfaces or block access to the stigma. Stigmas, depending on floral architecture, may also create a barrier to pollen access or redirect released pollen. However, in species that exhibit at least some degree of self-compatibility, it is logical to conclude that the spatial separation of anthers and stigmas will reduce the likelihood of self-fertilisation (Webb and Lloyd 1986).

Large-scale work on floral evolution within angiosperms have predicted early floral structures (Sauquet *et al.* 2017), but potential evolvability (capacity to adapt to changing conditions or pressures) of herkogamy, was calculated to be high and rapid in response to environmental changes and that the high degree of herkogamic variation often encountered within related species and populations could result from adaptive responses to the pollination environment (Opedal *et al.* 2017). It was found that the median evolvability of herkogamy was an order of magnitude greater than that of

other floral measurements and was not strongly constrained by genetic covariance between pistil and stamen lengths. They concluded that herkogamy can rapidly evolve in response to environmental changes, suggesting that variation in herkogamy in related species and populations may result from variation in pollinator communities and other selective factors. More recent work (Opedal *et al.* 2022) suggests that the evolvability of certain floral traits varies throughout phenotypic space and that particular trait combinations may be subject to strong genetic constraints that stall evolution towards optimum self-pollination rates. This work on the potential genetic constraints of functionally interactive floral traits (Opedal *et al.* 2022) reinforces herkogamy's high level of independent evolutionary potential.

Effect of herkogamy on pollination success

Numerous studies have demonstrated negative relationships between herkogamy and rates of autofertility (seed set in the absence of pollinators) and self-fertilisation (Moeller 2006; Herlihy and Eckert 2007; Eckert *et al.* 2009; Dart *et al.* 2012; Opedal *et al.* 2016), speaking to the functional importance of the trait. Additional studies have demonstrated positive relationships between pollinator abundance and herkogamy (Moeller 2006; Opedal *et al.* 2016).

The separation of pollen-producing anthers from receptive stigmas can influence rates of self-pollination amongst plants (Müller 1883). The degree of stigma exertion (relative to anthers) has a major influence on the efficacy of self-pollination in many types of plants and is mainly determined by the relative lengths of anthers, styles and ovaries (Shang *et al.* 2021). Reducing herkogamy can lead to improved rates of successful self-pollination in species that are self-compatible (Luo and Widmer 2013; Toräng *et al.* 2017; Opedal 2018). Logically, reducing the space between anthers and stigmas may lead to increased or enhanced self-pollen transfer during both biotic and abiotic-induced floral movements. Approach and reverse herkogamy have been shown to have considerably different effects on pollen loads, outcrossing rates and seed set (Barrett 2002). Approach herkogamous flowers were shown to have captured less total pollen but exhibited much higher rates of outcrossing than reverse herkogamous flowers, supporting Webb and Lloyd's (1986) assumption that approach herkogamy was superior in preventing self-pollination.

Predominately, studies that have investigated herkogamy-outcrossing or herkogamy-autofertility relationships have demonstrated that more herkogamous individuals, populations, and species tend to be more outcrossed (reviewed by Opedal 2018). The effect of greater anther-stigma separation on reducing individual selfing rates has been established for numerous taxa (Rick *et al.* 1978; Brunet and Eckert 1998; Motten and Stone 2000; Takebayashi *et al.* 2006).

Takebayashi *et al.* (2006) demonstrated a strong positive association between herkogamy and outcrossing rates in *Gilia achilleifolia*, which supports previous findings. Certainly, within populations, flowers exhibiting greater herkogamy do often tend to be more outcrossed (Opedal 2018). This can result from reduced interference between anther and stigmas when they are highly separated. In self-incompatible species, reduced herkogamy could lead to pollinators being restricted from stigmas or lead to an increase in self-pollen deposition leading to clogged stigmas or increasing competition between self- and cross-pollen (Fægri and Van der Pijl 1979; Opedal 2018).

Variation of herkogamy within species

Herkogamy can exhibit significant variation among genotypes within a species and has been proposed to show local adaptation to the pollination environment (Opedal 2018). In tomato (*Solanum lycopersicum*), this variation is evident, particularly between self-incompatible wild relatives and autogamous domesticated varieties.

Among wild tomato relatives such as *Solanum pennellii* and *Solanum peruvianum*, exerted stigmas and allogamous breeding are prevalent traits (Rick and Lamm 1955; Rick 1982, Rick and Chetelat 1991). In contrast, commercially important domesticated varieties like *S. lycopersicum* exhibit reverse herkogamy, where stigmas are inserted within the anther cone, facilitating self-pollination (Rick and Lamm 1955; Rick *et al.* 1977; Karron *et al.* 1997; Motten and Stone 2000). The transition to inserted stigmas in domesticated tomatoes likely occurred alongside the development of self-compatibility during domestication (Georgiady and Lord 2002; Georgiady *et al.* 2002; Blanca *et al.* 2012).

Research by Liu *et al.* (2016) on *Primula* species demonstrated that reducing the distance between anthers and stigmas resulted in decreased outcrossed pollen deposition and increased self-pollen deposition. However, total pollen deposition remained relatively unaffected. Opedal (2018) further explored the qualitative relationships between herkogamy and mating systems, suggesting a negative association between herkogamy and autofertility, with potentially more pronounced declines in approach herkogamous species. However, the relationship between herkogamy and outcrossing rates remains challenging to elucidate due to complex interactions with pollinators (Opedal 2018).

Hormonal control of herkogamy

The growth and development of all tissues in plants are intricately regulated by the interplay of phytohormones. From the inception of floral primordia, auxin serves as a morphogenetic catalyst, facilitating the formation of primordia.

Subsequently, it collaborates with other hormones to orchestrate the intricate processes of development, patterning and functioning of reproductive organs (Dresselhaus and Schneitz 2014; Zažímalová *et al.* 2014).

Cytokinin plays a crucial role in gynoecium patterning, particularly in early cell proliferation and inducing medial tissues in developing gynoecia (Marsch-Martínez *et al.* 2012). Cytokinin positively regulates reproductive meristems, which are relevant to inflorescence architecture. It has been proposed that high auxin levels are necessary for stigma and style development, low auxin and cytokinin induce ovary and ovule development, and high cytokinin levels regulate gynophore formation. Moreover, auxin signalling during gynoecium development has been associated with brassinosteroid signalling (Marsch-Martínez and de Folter 2016).

Stamen development in *Arabidopsis thaliana* is influenced by auxin, gibberellic acid (GA) and jasmonic acid (JA) coordinating and regulating various aspects of stamen development (Song *et al.* 2013). This includes stamen primordia formation, filament and anther development, pre-anthesis filament elongation, pollen maturation and anther dehiscence. Auxin transport from the tapetum through the middle layer and toward the filament is essential for coordinating anther maturation with filament growth (Cecchetti *et al.* 2017). Auxin is also involved in regulating stamen number and outgrowth (Heisler *et al.* 2005).

Defects in GA biosynthesis or perception result in shortened stamen filaments (Cheng *et al.* 2004; Tyler *et al.* 2004; Rieu *et al.* 2008a, 2008b). GA induces the synthesis of JA. However, the short stamen phenotype of GA-deficient plants cannot be rescued by exogenous JA, suggesting the involvement of other GA-dependent, JA-independent pathways in correct stamen filament elongation (Cheng *et al.* 2009).

Mutations in JA biosynthesis genes or components of the JA signalling pathway affect filament elongation (Xie *et al.* 1998; Stintzi and Browse 2000; Ishiguro *et al.* 2001; Park *et al.* 2002). Moreover, the development of stamens, including filament elongation, anther dehiscence and pollen maturation, is regulated by various environmental cues and endogenous hormone signals, including GA and JA (Ma 2005; Plackett *et al.* 2011; Song *et al.* 2013).

In species exhibiting distyly, local changes in genes associated with brassinosteroids have been attributed to the control of stamen and style length (detailed below). The application of brassinosteroids to primrose S-morphs caused an increase in style length, but not stamen length (Huu *et al.* 2016).

Genetic control of herkogamy

As herkogamy is determined by the positioning of anthers and stigmas in the flower, there is a need to understand the

molecular control of growth and development of the gynoecium and stamens (filaments and anthers). The molecular regulation of growth and development of these floral structures have been extensively reviewed (Herrera-Ubaldo *et al.* 2018, 2023; Thomson and Wellmer 2019) so will not be covered in detail here. Key to the determination of the gynoecium and stamens are the floral homeotic B, C and E function genes. The C function gene *AGAMOUS* (*AG*) together with the E function genes *SEPALLATA* (*SEP*) determine gynoecium development, and *AG* together with *PISTILLATA* (*PI*), *APETALA3* (*AP3*) and *SEP* genes determine stamen development.

This review will focus on genes that have been reported to alter the differential length of these organs with respect to herkogamy. As may be expected, many of these genes are associated with the hormones detailed in the section above. It is well documented that hormones work together to control different aspects of development, and this seems to be the case in the control of gynoecium length and stamen length. As the genetic control of the gynoecium and stamen are independently controlled, for this review we will examine the molecular control of these organs separately.

Molecular control of gynoecium development

Variability in gynoecium length may be determined by ovary length, or, more commonly, style length. The gynoecium in the model plant *Arabidopsis* is predominantly ovary tissue

with very little style tissue. However, there are a number of mutations associated with stylar tissue (Table 2, Fig. 3a). Genes associated with *Arabidopsis* ovary length appear to be key regulators of herkogamy with an E3 ubiquitin ligase gene, specifically *ARM-Repeat Containing1* (*ARC1*), being shown to control ovary length among different *Arabidopsis* ecotypes and promote approach herkogamy in *Arabidopsis* flowers (Indriolo *et al.* 2014). In other model species, such as *S. lycopersicum*, the style comprises the majority of the gynoecium length.

Style development appears to be associated with marginal tissue development, with the style positioned at the distal end of the gynoecium. Disruption of marginal tissue and organ fusion often leads to a loss of stylar tissue. The MADS-box transcription factor *SHATTERPROOF* (*SHP*) (Colombo *et al.* 2010) and other key transcription factors *AUXIN RESPONSE FACTOR6/8* (*ARF6/8*), *CRABSCLAW* (*CRC*), *AINTEGUMENTA* (*ANT*), *FILMENTOUS FLOWER* (*FIL*), *SUESS* (*SEU*), *LUNIG* (*LUG*) and *SPATULA* (*SPT*) have been characterised through mutation studies and shown to be associated with marginal tissue and style development. In cases where these genes are mutated individually or in combination, *Arabidopsis* gynoecia fail to fuse and often lack stylar and/or stigmatic tissue (Bowman *et al.* 1999; Liu *et al.* 2000; Alvarez and Smyth 2002; Groszmann *et al.* 2008).

The *STYLISH1* (*STY/SHI*) gene family comprises zinc-finger transcription factors expressed in the gynoecium, ovule primordia and style. Their status as downstream genes has been established through genetic investigations, wherein

Table 2. Genes involved in ovary and style length.

Organism	Gene	Gene model number	Gene class	Function	References
<i>Brassica rapa</i>	<i>ARM Repeat Containing1</i> (<i>ARC1</i>)	<i>LOC103865322</i>	U-box domain-containing protein	Controls ovary length; promotes approach herkogamy in <i>A. thaliana</i>	Indriolo <i>et al.</i> (2014)
<i>Arabidopsis thaliana</i>	<i>STIGMA AND STYLE</i> <i>STYLIST</i> (<i>SSS</i>)	<i>AT1G56020</i>	Unknown gene; serine/arginine repetitive matrix-like protein	Suppresses style elongation	Li <i>et al.</i> (2020)
<i>A. thaliana</i>	<i>NGATHA1</i> (<i>NGA1</i>)	<i>AT2G46870</i>	AP2/B3-like transcriptional factor	Involved in stigma development and style elongation	Alvarez <i>et al.</i> (2009) and Fourquin and Ferrándiz (2014)
<i>Solanum lycopersicum</i>	<i>Long styles</i> (<i>Lst</i>)	<i>Solyc12g027610.1</i>	Ethylene receptor EIN4-like	Inhibitor of style exertion	Cheng <i>et al.</i> (2021)
<i>S. lycopersicum</i>	<i>Style2.1</i> (<i>Se2.1</i>)	<i>Style21</i>	Polypeptide bearing a helix-loop-helix; transcription factor	Promotes style growth	Chen <i>et al.</i> (2007)
<i>S. lycopersicum</i>	<i>Stigma exertion 3.1</i> (<i>SE3.1</i>)	<i>Solyc03g098070</i>	C2H2 zinc finger transcription factor	Regulates anther and style length to control flush stigma development	Shang <i>et al.</i> (2021)
<i>S. lycopersicum</i>	<i>Woolly</i> (<i>Wo</i>)	<i>Solyc02g080260</i>	HD ZIP (IV)	Regulates the expression of <i>SE2.1</i>	Wu <i>et al.</i> (2024)
<i>Oryza sativa</i> subsp. <i>japonica</i>	<i>GRAIN SIZE 3</i> (<i>GS3</i>)	<i>OSNPB_030407400</i>	M-type_MADS	Regulates stigma length and exertion	Takano-Kai <i>et al.</i> (2011)
<i>Primula forbesii</i>	<i>CYP734A50</i>	<i>AOA32958</i>	Cytochrome P450	Inactivates brassinosteroid mediated growth activity	Huu <i>et al.</i> (2016)
<i>Turnera subulata</i>	<i>TsBAHD</i>	<i>QDPI6902</i>	Cytochrome P450	Homologous to <i>CYP73A50</i> similar to above	Shore <i>et al.</i> (2019)

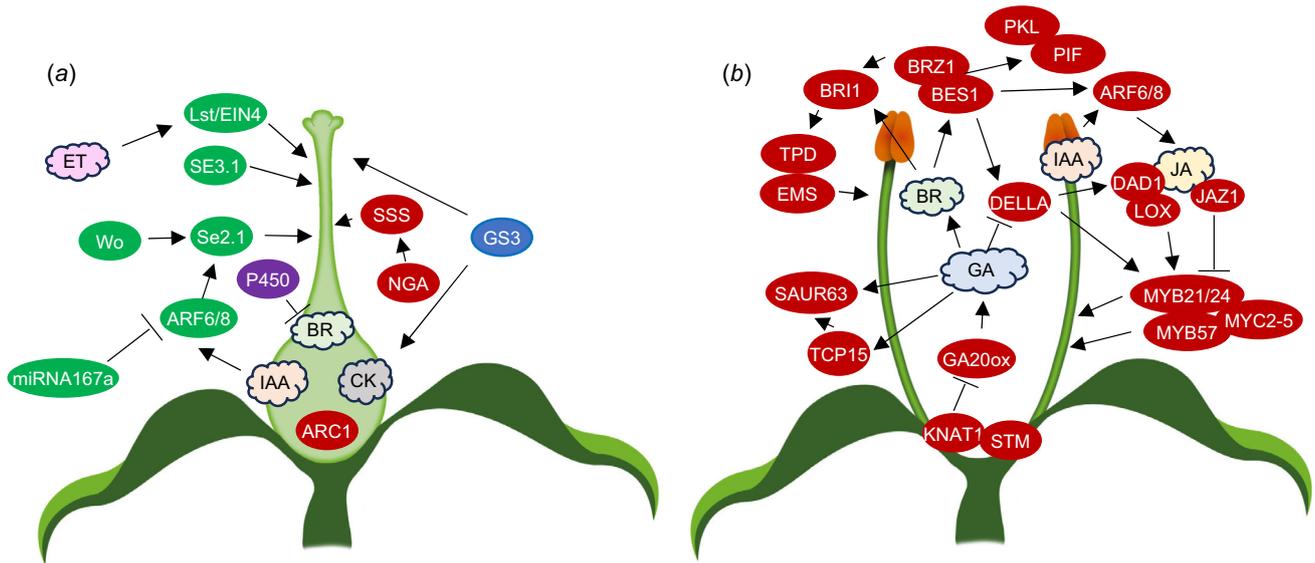


Fig. 3. Genes and hormones shown to control (a) ovary and style length and (b) filament length. Ovals, genes; red, *Arabidopsis*; green, tomato; blue, rice; purple, *Primula/Turnera*; clouds, hormones; IAA, auxin; BR, brassinosteroids; CK, cytokinins; ET, ethylene; JA, jasmonic acid; GA, gibberellins; arrows, positive reported relationships; lines with bars, negative reported relationships.

lug mutations were found to be epistatic over *sty* mutations, and *lug* mutants exhibited reduced expression of *STY1* (Kuusk *et al.* 2006). Ectopic expression of *STY1* rescues style defects of *lug*, *seu*, *crc*, *jag* (*JAGGED*) and *spt* mutant gynoecia (Ståldal *et al.* 2008). Supporting the downstream status of *STY1* from these genes, *sty1* mutants result in only minor style development defects compared with mutations in the upstream genes (Sundberg and Ferrándiz 2018). The auxin biosynthesis gene *YUCCA4* (*YUC4*) is among the downstream targets of *STY1*, indicating that *SHI/STY* family genes have a positive regulatory effect on local auxin biosynthesis (Sohlberg *et al.* 2006; Eklund *et al.* 2010). Supporting this, the application of exogenous auxin on the apical region of immature *sty1 sty2* mutant gynoecia leads to a reversion to the wild-type style and stigma (Sundberg and Ferrándiz 2018).

Once patterning has been established, the control of style length has been studied using mutants in *Arabidopsis* and by gene mapping in other species. The *Arabidopsis* *NGATHA* (*NGA*) B3-domain transcription factors (Alvarez *et al.* 2009; Trigueros *et al.* 2009) serve as redundant regulators influencing the establishment of apical gynoecium and stylar length. Inactivation of individual or multiple *NGA* members leads to elongated and misshapen stylar tissue. Fourquin and Ferrándiz (2014) identified *NGA* orthologues in the longer-styled species *Eschscholzia californica* (Californian poppy) and *Nicotiana benthamiana*. Expression patterns of *EcNGA* and *NbNGA* genes closely resembled *Arabidopsis* *NGA*. Inactivation of these genes caused severe defects in style. Downstream of the *NGA* transcription factors are the *STIGMA AND STYLE STYLIST* (*SSS*) genes (Li *et al.* 2020), which encode a novel class of angiosperm-specific genes

with unknown function. *Arabidopsis* possesses three partially redundant *SSS* genes with slightly different expression zones. Similar to *nga* mutants, suppression of *SSS* genes leads to elongated stylar tissue, whereas overexpression of *SSS* causes significant reduction in stylar length. Moreover, *SSS* overexpression effectively rescues the stylar defect in *nga1nga3* double mutants, affirming the pivotal role of *NGA* transcription factors in regulating these genes.

Genetic investigations into stigma position in tomato have revealed a quantitative determination influenced by relatively few genes with high heritability and dominance effects (Rick and Dempsey 1969). One major quantitative trait locus (QTL) named *style2.1* (*se2.1*) was identified on chromosome 2 (Chr2) using an interspecific mapping population derived from *S. lycopersicum* and *Solanum habrochaites* (Bernacchi and Tanksley 1997). The gene responsible for *se2.1* was subsequently characterised and found to encode a polypeptide with a helix-loop-helix (HLH) motif, but lacking the typical basic region found in the broader class of basic-helix-loop-helix (bHLH) transcription factors (Chen *et al.* 2007). The *SE2.1* protein promotes cell elongation in the style. The effect of the *se2.1* allele can be attributed to a deletion in the promoter region and a resultant decrease in gene transcription in cultivated tomato, explaining part of the transition from long to short styles (Chen *et al.* 2007; Vosters *et al.* 2014). *SE2.1* expression has been shown to be controlled by three HD-ZIP proteins, *WO*, *HD7*, *HD7L*. A dominant mutation of *WO* (*wo*^{W106R}) causes further style-length reduction to create reverse herkogamy (Wu *et al.* 2024). Additional QTLs for style exertion have been identified through interspecific biparental populations.

Three QTLs were reported on Chr4, Chr8 and Chr9 using a cross with *Solanum pimpinellifolium* (Georgiady *et al.* 2002), and one QTL was found on the long arm of Chr5 using a cross with *S. habrochaites* (Gorguet *et al.* 2008). Another locus, SE3.1, was identified through a genome-wide association study (GWAS) (Shang *et al.* 2021). This study identified a mutation in a C2H2 zinc finger transcription factor that plays a role in controlling stylar length. The *long styles* (*Lst*) phenotype in *S. lycopersicum* was suggested to be linked to an *EIN4*-like ethylene receptor (Solyc12g027610.1), implicating ethylene in conferring this style exertion phenotype (Cheng *et al.* 2021).

Riccini *et al.* (2021) demonstrated a significant involvement of genes related to indole-3-acetic acid (IAA) function in exerted stigma genotype groups in tomato, supporting the accepted role of auxin in the apical-basal patterning development of the gynoecium in *Arabidopsis* (Ståldal and Sundberg 2009). In tomato, downregulation of *ARF6* and *ARF8* via miRNA167 overexpression resulted in shortened floral organs compared with control plants (Liu *et al.* 2014). This phenotype was accompanied by a decrease in SE2.1 expression, suggesting that *ARF6* and *ARF8* may function upstream of SE2.1, and the reduced expression of SE2.1 in MIR167a-plants contributes to reduced style growth (Liu *et al.* 2014).

In rice (*Oryza sativa*), *GRAIN SIZE 3* (*GS3*), a MADS box gene known for regulating seed length, has been found also to influence style and (non-brush) stigma length resulting in stigma exertion (Takano-Kai *et al.* 2011). Its basal mRNA expression is detected in young rice stigmas, and a nonsense mutation in its second exon leads to cell proliferation, resulting in elongated stigmas.

In distylous species, both the stamen and the style length are controlled by an 'S-locus' containing several genes recently reviewed (Kappel *et al.* 2017). In *Primula* (Huu *et al.* 2016, 2022) and *Turnera* (Shore *et al.* 2019; Matzke *et al.* 2021), cytochrome P450 genes that are homologous to *Arabidopsis* CYP72B1 (*BAS1*) have been shown to control style length. *BAS1* modulates brassinosteroid associated growth (Neff *et al.* 1999) providing a possible mechanism for how this class of gene can regulate different stylar morphs (Henning *et al.* 2020).

In the mangrove species *Lumnitzera littorea*, Zhang *et al.* (2020) identified 12 MADS-box genes, including four MIKC-type and eight M-type transcription factors, associated with herkogamy. Some species exhibit stylar polymorphisms, leading to individuals with either approach or reverse herkogamy. Several major loci influencing such polymorphisms have been identified in genetic studies (Barrett *et al.* 2000; Ushijima *et al.* 2012; Nowak *et al.* 2015). Additionally, in other species, style length exhibits continuous variation, resulting in individual flower profiles ranging between approach and reverse herkogamy (Motten and Stone 2000; Takebayashi *et al.* 2006). The regulation of style length and stamen position often involves multiple loci with minor

effects, and covariation between floral organs is not uncommon (Conner and Sterling 1996; Herrera 2001; Kulbaba and Worley 2008). Lande and Arnold (1983) suggest this continuous covariation could limit the evolvability of herkogamy, in contrast to earlier studies that proposed it to be highly evolvable (Opedal *et al.* 2017).

In an RNA-seq comparison of inserted- and exerted-stigma tomato genotypes (Riccini *et al.* 2021), the exerted genotypes showed an over-representation of genes involved in regulating cell wall structure and metabolism. Notably, two xyloglucan endotransglucosylase-hydrolases (*XTH1* and *XTH7*), three expansins or expansin-precursor genes (*EXP5*, *EXP12* and *EXPA4*), genes related to sucrose metabolism (*STD1*, *SPS*, *SUSY*), and the auxin efflux facilitator *PIN-FORMED3* (*PIN3*) were identified among the cell wall-related genes in the exerted genotypes.

Molecular control of stamen development

Stamens, consisting of filaments and anthers and collectively known as the androecium, produce male gametophytes (D'Arcy 1996). The floral homeotic gene *AG* regulates stamen specification in part through JA genes (Bowman *et al.* 1991; Sieburth and Meyerowitz 1997). *AG* directly regulates *DEFECTIVE IN ANTHWER DEHISCENCE1* (*DAD1*), a JA catalytic enzyme, and controls stamen identity and differentiation through transcriptional cascades at different floral stages, including the regulation of *SPOROCTYLESS/NOZZLE* (*SPL/NZZ*) and JA biosynthesis (Ito *et al.* 2004, 2007).

For correct stamen development in *Arabidopsis* (Table 3, Fig. 3b), repression of Class I *KNOX* genes must occur during early flower development (Tabata *et al.* 2010; Rubio-Somoza and Weigel 2013). At late pre-anthesis, stamen growth typically increases rapidly before decreasing as the stamen reach the top of the pistil (Tashiro *et al.* 2009). In post-anthesis stages, it has been suggested that *KNAT1* works redundantly with (*SHOOTMERISTEMLESS*) *STM* to limit stamen growth (Gastaldi *et al.* 2023). *KNAT1* overexpression was shown to strongly reduce filament elongation. Defective GA synthesis causes reduced filament elongation (Rieu *et al.* 2008b). *KNAT1* overexpression decreases transcription levels of the GA biosynthesis gene *GA20OX1*, indicating filament elongation may be repressed by *KNAT1* via regulation of GA biosynthesis (Gastaldi *et al.* 2023). Late filament elongation is stimulated by members of the TCP transcription factor family and *KNAT1* directly targets *TCP15* to modulate filament elongation (Gastaldi *et al.* 2020, 2023). Class I TCPs are needed for the GA induction of *SMALL AUXIN UP RNA 63* (*SAUR63*) subfamily genes. SAUR proteins promote cell expansion by activating plasma membrane H⁺-ATPases (Spartz *et al.* 2014) and overexpression of *SAUR63* subfamily members stimulates stamen filament elongation (Chae *et al.* 2012). *SAUR63*

Table 3. Genes involved in filament length in *Arabidopsis thaliana*.

Organism	Gene	Gene model number (Locus tag)	Gene class	Function/Phenotype	References
<i>Arabidopsis thaliana</i>	BREVIPEDICELLUS/ KNATI	AT4G08150	Class 1 knotted-like homeobox gene	Represses TCP15 to limit filament growth	Gastaldi <i>et al.</i> (2020)
<i>A. thaliana</i>	SHOOT MERISTEMLESS (STM)	AT1G6230	KNOX/ELK homeobox transcription factor	Works redundantly with KNATI to restrict stamen growth	Gastaldi <i>et al.</i> (2023)
<i>A. thaliana</i>	REPRESSOR OF GA/ RGA-like2 (RGA)/(RGA2)	AT2G01570/ AT3G03450	GRAS family TF; DELLA protein	Inhibitors of stamen growth	Cheng <i>et al.</i> (2009)
<i>A. thaliana</i>	TCP15	AT1G69690	TCP family TF	Activates SAUR63 in filament elongation	Gastaldi <i>et al.</i> (2020, 2023)
<i>A. thaliana</i>	DAD1	AT2G44810	alpha/beta-Hydrolases superfamily protein	Anther dehiscence; catalyses the initial step of jasmonic acid biosynthesis.	Ishiguro <i>et al.</i> (2001)
<i>A. thaliana</i>	MYB21/MYB24	AT3G27810/ AT5G40350	TF; MYB domain protein 21/24	Differentiation and development of stamen. Induced by jasmonate.	Mandaokar <i>et al.</i> (2006)
<i>A. thaliana</i>	MYB57	AT3G01530	TF; R2R3 factor gene family	Specifies stamen identity and filament length	Cheng <i>et al.</i> (2009)
<i>A. thaliana</i>	BZR1/BES1 (BRASINOSTEROID-RELATED GENES)	AT1G75080	Brassinosteroid signalling positive regulator (BZR1) family protein	Influence cell elongation and stamen size	Chen <i>et al.</i> (2019)
<i>A. thaliana</i>	SMALL AUXIN UP RNA 63 subfamily (SAUR63)	AT1G29440	SAUR-like auxin-responsive protein family	Overexpression restores filament growth	Gastaldi <i>et al.</i> (2020)
<i>A. thaliana</i>	LIPOXYGENASE (LOX1)	AT1G55020	Lipoxygenase 1; protein coding JA-related gene	Activates MYB TFs to mediate filament elongation	Cheng <i>et al.</i> (2009)
<i>A. thaliana</i>	Jasmonate zim domain protein 1 (JAZ1)	AT1G19180	Jasmonate signalling nuclear localised protein	Represses MYB21 and MYB24 to inhibit stamen filament elongation	Huang <i>et al.</i> (2020)
<i>A. thaliana</i>	AUXIN RESPONSE FACTOR6/8 (ARF6/8)	AT1G30330 (ARF6) AT5G37020 (ARF8)	ARF transcription factors	Induce expression of JA biosynthesis in stamen development; ARF8.4 variant needed for filament elongation	Ghelli <i>et al.</i> (2023)
<i>A. thaliana</i>	EMS1/EXS (EXCESS MICROSPOROCYTES1)	AT5G07280	Leucine-rich repeat transmembrane protein kinase	Regulates somatic and reproductive cell fates in anther development	Bai <i>et al.</i> (2023)
<i>A. thaliana</i>	BRASINOSTEROID INSENSITIVE 1 (BRI1)	AT4G39400	Leucine-rich receptor-like protein kinase family protein	Regulates filament elongation via the downstream activity of BZR1/BES1	Bai <i>et al.</i> (2023)

overexpression in plants expressing a fusion of *TCP15* to a repressor domain (*pTCP15::TCP15-EAR*) rescued short stamens whilst *TCP15* overexpression did the same in GA-deficient plants (Gastaldi *et al.* 2020).

In *Arabidopsis*, DELLA proteins play a crucial role in regulating phytohormones for filament elongation (Huang *et al.* 2020). Cheng *et al.* (2009) demonstrated that DELLA proteins REPRESSOR OF GA (*RGA*) and *RGA*-like2 (*RGL2*) were principal players in inhibition of stamen development. GAs suppress DELLA proteins and upregulate the JA-related genes *DAD1* and *LIPOXYGENASE (LOX1)* to activate *MYB21*, *MYB24* and *MYB57* transcription factors to mediate filament elongation (Cheng *et al.* 2009). Huang *et al.* (2020) further demonstrated that DELLAs directly targeted *MYB21* and *MYB24* that govern GA-JA interaction in filament elongation and that co-expression of DELLA (*RGL2*) and *JASMONATE ZIM*-domain (*JAZ1*) proteins repressed *MYB21* and *MYB24* to inhibit filament elongation.

Mutations in genes associated with auxin, specifically *AUXIN RESPONSE FACTOR* genes *ARF6* and *ARF8*, lead to deficiencies in stamen filament elongation and anther maturation (Nagpal *et al.* 2005; Cecchetti *et al.* 2008; Tashiro *et al.* 2009; Tabata *et al.* 2010; Reeves *et al.* 2012). Specifically, a splice variant of *ARF8* (*ARF8.4*) is required for stamen filament elongation and correct expression of the auxin-inducible gene *AUX/IAA19* (Ghelli *et al.* 2023). *ARF6* and *ARF8* have been reported to induce the expression of JA biosynthesis genes during late stages of stamen development, suggesting that auxin acts upstream of JA (Nagpal *et al.* 2005; Tabata *et al.* 2010; Song *et al.* 2011; Reeves *et al.* 2012). In *arf6 arf8* double null mutants, plants produce short filaments and un-dehisced anthers whilst delayed filament elongation is observed in *arf6* and *arf8* single mutants homozygous for one mutation and heterozygous for the other (Nagpal *et al.* 2005; Wu *et al.* 2006; Tabata *et al.* 2010; Reeves *et al.* 2012). However, the fact that

stamen filament elongation is not rescued by JA treatment in *arf6 arf8* mutants (Nagpal *et al.* 2005) indicates the involvement of additional pathways. Among the genes downregulated in *arf6 arf8* mutant flowers, several previously mentioned SAUR genes from the SAUR63 subfamily were identified (Nagpal *et al.* 2005). Therefore, the induction of SAUR genes by ARF6 and ARF8 may be required, in addition to JA biosynthesis, to stimulate filament elongation as JA synthesis induces expression of MYB21 and MYB24 (Reeves *et al.* 2012).

Qi *et al.* (2015) previously demonstrated that transcription factors MYC2–5 were JAZ-targeted proteins that worked redundantly to regulate filament elongation and formed a complex with MYB21 and MYB24 in filament elongation in *Arabidopsis*. In *myb21* null mutants, filament elongation is prevented (Mandaokar *et al.* 2006) and anther opening delayed but pollen remains viable, whilst in *myb21 myb24* double mutants filament elongation, anther dehiscence and pollen viability are all affected, suggesting MYB21 is essential for filament elongation and works redundantly with MYB24 for anther dehiscence and pollen viability (Mandaokar *et al.* 2006; Reeves *et al.* 2012; Acosta and Przybyl 2019).

The brassinosteroid-related gene *BZR1* and its close homologue *BES1* (*BZR1/BES1*) influence cell elongation to affect stamen length (Chen *et al.* 2019). DELLAs, PIFs, ARF6, and PKL directly interact with *BZR1/BES1* to form a cell elongation regulatory network (Li *et al.* 2018). *BZR1/BES1*'s transcriptional activity can be constrained by the direct physical interaction of DELLAs leading to restrained cell elongation (Li *et al.* 2012). Also involved in influencing stamen development in *Arabidopsis* are *EMS1/EXS*. Bai *et al.* (2023) reported the transgenic expression of brassinosteroid receptor *BRI1* rescued short filaments in *ems1* mutants that were deficient in stamen elongation whilst co-expression of *EMS1* and *TPD1* rescued short filaments in *bri1* mutants (Bai *et al.* 2023). Subsequently, *EMS1* and *BRI1* regulate filament elongation via the downstream activity of *BZR1/BES1* (Bai *et al.* 2023).

Environmental effects on herkogamy

Herkogamy is subject to the influence of various environmental factors in different plant species. Environmental factors, such as water relations, temperature and light quality, have been shown to influence herkogamy by affecting floral development. In *Arabidopsis*, temperature and genotype have been suggested as determinants of herkogamy (Luo and Widmer 2013). Similarly, in tomato, style elongation has been linked to genotype dependence and sensitivity to relatively high temperatures (Peralta *et al.* 2005). Saeed *et al.* (2007) found that the length of the style of different tomato genotypes increased by 25–55% under high temperatures, whilst Sakata *et al.* (2010) demonstrated that auxin levels in tomato decreased under high temperature conditions

leading to shorter filaments. The possibility of the opposite effect on pistils under high temperatures, resulting in stigma exertion, has been proposed (Riccini 2019; Riccini *et al.* 2021). Additionally, it was observed that higher temperatures were needed to cause tomato stigma exertion in the ST1st1 mutation (Cheng *et al.* 2021).

In the high-altitude plant *Aquilegia coerulea*, Van Etten and Brunet (2013) observed phenotypic plasticity in herkogamy in response to changes in both water availability and temperature. Plants subjected to higher water treatments exhibited longer styles, while stamen lengths remained unchanged. In contrast, higher temperatures decreased the length of both styles and stamens, resulting in varying effects on herkogamy across different study populations in Colorado, Utah and Arizona with no change, a decrease, and an increase in herkogamy, respectively.

In a study on *Arabidopsis* and *Brassica* floral organs in response to light quality, Weinig (2002) found that wild-type *Arabidopsis* exposed to low red:far-red (R:FR) light had diminished petal and pistil lengths but longer filaments relative to petal size compared to those exposed to high R:FR light. Similar results were observed for *Brassica*. *Arabidopsis* mutants lacking phytochrome A exhibited smaller petals, pistils, and filaments, while those deficient in phytochrome B had longer filaments (Weinig 2002).

Concluding remarks

Herkogamy is an important floral trait that plays a crucial role in promoting cross-pollination and maintaining genetic diversity in plant populations. The genetic and hormonal control of herkogamy is a complex and dynamic process that involves multiple genetic pathways and environmental factors. Abiotic factors such as water availability, temperature, and light quality also play significant roles in modulating floral development and affecting herkogamy.

Recent advances in genomic and molecular techniques have greatly expanded our understanding of the genetic and hormonal regulation of herkogamy. These advances have helped to identify key genes and pathways that control herkogamy and have shed light on the molecular mechanisms underlying the development and maintenance of the trait.

However, there is still much to learn about the genetic and hormonal control of herkogamy, particularly in non-model plant species. Future research should focus on identifying additional genes and pathways involved in herkogamy regulation, as well as exploring the role of environmental factors and epigenetic modifications in herkogamy development.

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