Heterochronic Developmental Shifts Underlie Floral Diversity within *Jaltomata* (Solanaceae)

Jamie L. Kostyun[1*], Jill C. Preston[2,a], and Leonie C. Moyle[1,b]

[1] *Department of Biology, Indiana University, Bloomington, Indiana 47405, USA*

[2] *Department of Plant Biology, The University of Vermont, Burlington, Vermont 05405, USA*

[a] Jill.Preston@uvm.edu

[b] lmoyle@indiana.edu

*Corresponding author: jkostyun@indiana.edu

Abstract

Background: Heterochronic shifts during mid to late stages of organismal development have been proposed as key mechanisms generating phenotypic diversity. To determine whether late heterochronic shifts underlie derived floral morphologies within *Jaltomata* (Solanaceae) – a genus whose species have extensive and recently evolved floral diversity – we compared floral development of four diverse species (including an ambiguously ancestral or secondarily derived rotate, two putatively independently evolved campanulate, and a tubular morph) to the ancestral rotate floral form, as well as to an outgroup that shares this ancestral floral morphology.

Results: We determined that early floral development (<1 mm bud diameter, corresponding to completion of organ whorl initiation) is very similar among all species, but that different mature floral forms are distinguishable by mid-development (>1 mm diameters) due to differential growth acceleration of corolla traits. Floral ontogeny among similar mature rotate forms remains comparable until late stages, while somewhat different patterns of organ growth are found between species with similar campanulate forms.

Conclusions: Our data suggest shared floral patterning during early-stage development, but that different heterochronic shifts during mid- and late-stage development contributes to divergent floral traits. Heterochrony thus appears to have been important in the rapid and repeated diversification of *Jaltomata* flowers.

Keywords: Convergent Evolution, Flower development, Heterochrony, *Jaltomata*, Scanning electron microscopy, Solanaceae.

Background

In The Origin of Species, Darwin stated "we can actually see in embryonic crustaceans and in many other animals, and in flowers, that organs, which when mature become extremely different, are at an early stage of growth exactly alike." [1]. This prescient statement both suggests the importance of understanding diversity in a developmental and phylogenetic framework [2], and raises the question about which mechanisms underlie these hypothesized mid to late stage shifts in ontogeny. Heterochrony—a change in the relative timing or rate of a developmental process between a derived lineage and its ancestor—is one potential mechanism contributing to such trait variation [3-4]. Since being formally defined by Haeckel [5], the concept of heterochrony has undergone significant changes in its usage and application [6-9; reviewed in 10-11]. Despite these differences in the exact definition utilized, heterochrony is still broadly viewed as an important framework in which to examine and understand morphological changes [3; 10], especially in animal taxa. For instance, the classic example of axolotls that retain more juvenilized features than their ancestors [12], the large variation in cranial morphology among different dog breeds [13], and even the evolution of extremely complex traits such as metamorphosis in insects [14], have largely been attributed to heterochronic shifts during development.

The general concept of heterochrony, as well as the distinct processes that comprise this mechanism of developmental change, can also be meaningfully applied to plant evolution [15-16]. Heterochrony is most often identified in plants by examining individual organs or functional units, such as flowers or individual leaves [4]. Broadly, heterochronic shifts include changes in the relative timing of initiation (i.e. onset) or termination (i.e. offset) of a developmental process – which together determine the duration of this developmental process. Additionally, changes

can occur in the rate at which a developmental process proceeds. Shorter developmental duration or decreased growth rate (termed pedomorphosis) often results in the reduction or juvenilization of a trait, while longer developmental duration or increased growth rate (termed peramorphosis) often results in the elaboration of a trait. Both types of shifts – duration or rate, as well as their combined effects – can conceivably contribute to observed variation in plant traits [3-4, 17].

Unlike plant architecture in general, flowers undergo determinate development, and thus are particularly attractive subjects for studying the contribution of heterochrony to morphological divergence. While most flowers conform to a shared ground plan, i.e. egg and pollen-producing structures surrounded by a non-reproductive perianth (sepals and petals), many lineages exhibit abundant inter- and intra-specific variation in the organization (phyllotaxy), number (merosity), size and shape, degree of fusion, and specific identity of floral organs [18-19]. Numerous studies have examined the potential role of heterochronic changes in floral trait evolution, including those associated with mating system transitions. The shift from outcrossing to predominately selfing is considered one of the most common evolutionary transitions among plants [20-21], and is typically associated with reduction of flower size, petal size, and/or anther-stigma separation (herkogamy) – collectively referred to as the 'selfing syndrome' [22-23]. Comparative work examining closely related outcrossing and selfing species, as well as among intra-specific populations [24], has generally revealed that reductions in floral size result from pedomorphic changes, either from decreased growth rates (i.e. neoteny; e.g. [25]), or truncation of the growth period [26]. However, some studies have also found that smaller flowers or those with reduced herkogamy in selfing lineages result from an increased growth rate over either short [27] or long [28] developmental periods, as well as different growth patterns between populations with high selfing rates [24]. Similarly, comparative work examining the developmental basis of

93 cleistogamous flowers (where sexual maturation and self-fertilization occurs within closed buds)
94 also indicates a prominent role of heterochronic changes [29]. Such cleistogamous flowers have
95 been found to result from various combinations of heterochronic shifts, including decreased
96 organ growth rates and shortening of the duration of development (e.g. [30]), or decreased
97 growth rates of petals and stamens (e.g. [31]). In contrast however, Luo et al. [32] determined
98 that cleistogamous flowers in *Pseudostellaria heterophylla* result from induction of fewer organ
99 primordia (including complete loss of petal primordia initiation) than in chasmogamous (i.e.
100 open flowers) on the same individual. Thus, several distinct heterochronic (and in fewer reported
101 cases, non-heterochronic) processes can result in convergent floral phenotypes associated with
102 mating system transitions, such as selfing syndromes and cleistogamy.

103 Apart from developmental studies of mating system transitions, comparative ontogenetic
104 studies have examined floral trait variation likely related to pollinator shifts [33-35]. A classic
105 example is Guerrant's [33] examination of developmental differences between a pair of
106 *Delphinium* species – one bee-pollinated, the other hummingbird-pollinated. In that instance, the
107 derived hummingbird-pollinated flower results from an overall decreased growth rate
108 (pedomorphosis via neoteny), but also accelerated growth for an extended period (peramorphosis
109 via acceleration and hypermorphosis) specifically in the reward-providing nectariferous petals.
110 More recently, Armbruster et al. [35] described the evolution of buzz-pollinated flowers in
111 *Dalechampia* via neoteny, with sexual maturity of the anthers occurring at an earlier
112 developmental stage (i.e. similar to pre-anthesis ancestral buds) than in species with the post-
113 anthesis ancestral floral form (i.e. with opened and extended petals and sepals). Nonetheless,
114 despite this increasing attention to the role of heterochrony in plant development, several critical
115 questions remain, including how frequently heterochronic shifts underlie changes in floral form,

116 and whether convergent phenotypes may be the result of similar or divergent developmental
117 mechanisms.

118 *Jaltomata* (Solanaceae) includes approximately 60-80 species distributed from the
119 Southwestern United States to the Andean region of South America, with extensive and recently
120 evolved floral diversity [36-39]. Although the pollination biology of this genus has yet to be
121 formally evaluated, field observations (T. Mione and S. Leiva G., pers. comm.) reveal that
122 different species are visited by bees and hummingbirds. In addition, *Jaltomata* produce varying
123 amounts of floral nectar as a pollinator reward, unlike close relatives like *Solanum* [40-41].
124 Examination of corolla morphology variation within Solanaceae [41] reveals that the closest
125 lineages to *Jaltomata* (*Solanum*, *Capsicum*, and *Lycianthes*) all have predominately flattened
126 'rotate' corollas, suggesting that this rotate form is ancestral within *Jaltomata*. Indeed, ancestral
127 state reconstruction on a molecular phylogeny of 45 *Jaltomata* species [37] supports the rotate
128 corolla form as ancestral, and that both bell-shaped 'campanulate' and elongated 'tubular' flowers
129 are derived specifically within the genus (Fig. 1). It also suggests that several instances of the
130 independent evolution of either derived campanulate or tubular floral forms in different
131 *Jaltomata* species; in contrast, both campanulate and tubular forms generally appear to produce
132 more nectar than rotate forms ([42]; T. Mione, pers. comm.; J. L. Kostyun, unpublished).
133 Phylogenetic reconstruction with fewer species but additional loci [36, 38], as well as
134 phylogenomic reconstruction using whole transcriptomes (Wu, Kostyun, Moyle, unpublished),
135 also recover the major clades identified in [37] further supporting the inferences that rotate
136 corolla morphology is ancestral within the genus and that campanulate corollas in different
137 clades are likely convergent rather than homologous.

138 We hypothesized that both derived campanulate and tubular forms might represent
139 elaborated versions of the ancestral rotate form, specifically through variation in corolla (petal)
140 growth prior to flower anthesis (opening). We therefore assessed whether heterochronic changes
141 (specifically peramorphic changes such as longer duration of development and/or growth
142 acceleration, which often produce larger or more elaborated structures) underlie these transitions,
143 particularly during late stages of ontogeny [1-2, 4]. (Therefore, although heterochrony can also
144 refer to changes in the actual developmental events (i.e. sequence heterochrony; [11]), here we
145 are particularly interested in assessing changes in growth). In addition to evaluating the role of
146 heterochrony in the evolution of derived floral forms, we also expected that comparing floral
147 development between species with similar mature morphs--but unresolved phylogenetic
148 relatedness--would provide insight into their evolutionary origin [37] (Wu, Kostyun, Moyle,
149 unpublished). For instance, if similar mature forms result from similar developmental processes,
150 this could suggest re-use of similar pathways or a shared single origin. In contrast, if they result
151 from distinct developmental processes, this could indicate that these forms are independently
152 derived, or that these forms share a common evolutionary origin (i.e. are homologous) but
153 underwent developmental systems drift [43] during their divergence.

154 Given these considerations, our main goals in this study were to: 1) assess evidence for
155 heterochronic changes during floral development in species with derived campanulate and
156 tubular forms; 2) determine which type(s) of heterochronic changes are associated with specific
157 floral trait changes; and 3) assess whether putatively convergent forms might result from similar
158 or different developmental processes (i.e. are associated with the same or different types of
159 heterochronic shifts). To address these goals, we compared floral ontogeny of four *Jaltomata*
160 species with divergent corolla morphologies to a *Jaltomata* species with a rotate corolla that is

representative of the inferred ancestral state (i.e. plesiomorphic), as well as an outgroup species that also has an inferred plesiomorphic rotate corolla form.

Our findings support the inference that a combination of parallel and convergent allometric changes (i.e. shifts in the size of particular floral organs in relation to the entire flower) have given rise to floral variation in this group, and show that heterochronic shifts explain these changes. In particular, two types of peramorphic changes (extended duration of floral development and accelerated growth) predominately explain changes in corolla traits. Thus, peramorphism emerges as an important developmental mechanism controlling diversity of *Jaltomata* corolla forms. Finally, species with similar mature campanulate corollas follow similar but clearly not identical growth trajectories, consistent with phylogenetic inferences that these campanulate floral morphs have independent origins [37]; (Wu, Kostyun, Moyle, unpublished) (Fig. 1). In contrast, we found that species with similar mature rotate corollas follow nearly identical developmental trajectories (specifically, that overall bud and organ growth rates do not differ) until the very last stages of ontogeny, suggesting a single evolutionary origin of the rotate form in this case.

Methods

Plant materials and growth conditions

Field-collected seeds of our five target *Jaltomata* species were provided by Dr. Thomas Mione (Central Connecticut State University), and seed for one wild tomato outgroup (*Solanum pimpinellifolium*, accession LA1589) was obtained from the Tomato Genetics Resource Center at the University of California, Davis (Table S1). We selected this outgroup because *Solanum* is considered the sister genus to *Jaltomata* [36, 38], this species has an ancestrally representative

184 rotate corolla [41], floral development in this species has previously been characterized [26, 44],
185 and--as a wild species--its floral development has not been influenced by domestication (i.e.
186 compared to domestic tomato, *S. lycopersicum*). Our focal *Jaltomata* species included the
187 Central American species *Jaltomata darcyana* that has an rotate corolla representative of the
188 inferred ancestral corolla morphology, and four species found as natives exclusively in South
189 America: *Jaltomata calliantha* and *Jaltomata dendroidea* with putatively independently derived
190 campanulate corollas, *Jaltomata umbellata* with a tubular corolla, and *Jaltomata sinuosa* with a
191 rotate corolla that is ambiguously ancestral or secondarily derived (Fig. 1). Plants were cultivated
192 in the Indiana University Research Greenhouse, under standardized temperature (15-20°C) and
193 light (16-hour days) conditions. All plants were watered to field capacity on automatic driplines,
194 and received weekly fertilizer treatment.
195
196 **Floral development**
197 To estimate the duration of floral development and overall absolute floral growth rate, young
198 inflorescences were first identified when the largest bud was approximately 1 mm in diameter,
199 and then evaluated every second day until first anthesis (petal opening). In particular, we
200 evaluated and measured bud diameter on the largest (oldest) bud from at least three
201 inflorescences on three individuals per species, except for *J. dendroidea* for which we could only
202 measure buds from two individuals (three and two inflorescences per individual, respectively).
203 To assess relative organ growth during development, buds up to 4 mm in diameter were
204 collected into 70% ethanol or formalin-acetic acid-alcohol (FAA), dissected as required,
205 dehydrated through an ethanol series at room temperature, and incubated in a 4°C fridge over-
206 night. Samples were critical point dried (using a Balzers CPD030), mounted on aluminum stubs,

207 and sputter-coated with gold-palladium (using a Polaron E5100). They were then imaged using a
208 JEOL 6060 or a JEOL 5800LV scanning electron microscope (SEM) at the University of
209 Vermont or Indiana University, respectively. Morphological traits were measured on the
210 resulting bud micrographs using ImageJ [45]. For buds greater than 4 mm in diameter,
211 morphological traits were measured by hand on freshly collected samples using digital calipers.
212 To ensure that we were comparing organ sizes at the same developmental stage across species,
213 we assigned measured buds to discrete developmental stages based on morphological markers,
214 following [44] for which floral developmental stages were determined in the same accession of
215 *S. pimpinellifolium* as used here. This resulted in six distinct 'early' developmental stages, one
216 'mid' stage, and three 'late' stages, based on these morphological markers (Table 1).
217 Given the potential role of trichomes in regulating corolla shape during bud development
218 [46], we also determined the developmental timing of trichome initiation and maturation. In
219 particular, we assessed whether divergent corolla morphologies are associated with differences in
220 trichome patterning during floral development. Because our focal species also vary in nectar
221 volume per flower, and derived campanulate and tubular forms are generally associated with
222 greater nectar volume across the genus (T. Mione, pers. comm.; J. L. Kostyun, unpublished), we
223 determined the onset of nectar secretion using a 20x hand-lens. Our primary goal was to
224 determine whether this observed difference in nectar volume between divergent corolla forms is
225 associated with changes in the developmental timing of nectar secretion.
226

227 **Mature floral trait measurements**

228 Mature floral traits were measured with hand-held digital calipers on three flowers each for at
229 least three individuals per species, and included calyx diameter, sepal length, corolla diameter,

230 corolla depth, corolla fusion (i.e. length of the corolla tube), petal length, lobe length, stamen
231 length, anther length, ovary diameter, and style length (Fig. S1). To account for potential size
232 differences among flowers within an inflorescence, floral traits were only measured on the oldest
233 flower within each examined inflorescence. For *Jaltomata* species only (the outgroup does not
234 produce nectar), we also measured nectar volume per flower to the nearest 1 μL with a pipette.
235 To reduce potential environmental effects on nectar production, nectar volume per flower was
236 always measured during the early afternoon following watering. Trichome type(s) present on
237 sepals and petals was also scored using a 20x hand lens.
238
239 **Statistical analyses**
240 We used analyses of variance (ANOVA), followed by Tukey's honest significant difference
241 (HSD) post-hoc tests, to assess differences among species in three comparisons: on mature floral
242 traits; log-transformed floral organ size at discrete stages during development (as specified in
243 Table 1); and duration (i.e. number of days) of floral development.
244 To compare relative floral organ growth rates during development, we used linear
245 regression on log-transformed bud measurements. Because buds grow at different rates among
246 species (Table 1), we regressed organ sizes on bud diameter to account for overall floral size;
247 thus, we assessed whether there were significant interaction effects between bud diameter and
248 species as a measure of differential growth rates. We performed these analyses in two ways.
249 First, we used data from the entire course of floral development. However, because we
250 determined that species differences in bud growth (specifically, bud diameter) only occur once
251 all floral organs are initiated (i.e. starting in stage G, Table 1), we also performed these
252 regressions separately for two discrete groups of developmental stage ("Early" versus "Mid" +

253 "Late", in Table 1). This enabled us to more directly examine organ growth in relation to overall
254 bud growth; we present the latter set of analyses in the main text, and the former (i.e. over total
255 floral development) within Table S3 for comparison. Because we are most interested in potential
256 heterochronic shifts within *Jaltomata*, we used *J. darcyana* as the baseline in these analyses to
257 assess differences between ancestral and derived floral forms. However, since *J. darcyana*
258 flowers could have somewhat derived developmental trajectories (and the actual ancestor of all
259 five *Jaltomata* species is no longer extant), we also performed these analyses using either *S.*
260 *pimpinellifolium* (as another representative of the inferred rotate ancestral state) or *S.*
261 *pimpinellifolium* + *J. darcyana* (as a 'composite' representation of the inferred rotate ancestral
262 state – specifically, we treated these species as a single group) as the baseline. These analyses
263 returned qualitatively consistent results as our comparisons using *J. darcyana* (see Results), and
264 are provided in Table S3 for comparison. We also compared organ growth rates between the two
265 derived campanulate forms (i.e. *J. calliantha* versus *J. dendroidea*). All analyses were performed
266 within the R statistical environment [47].
267

268 **Results**

269 **Mature floral traits differ markedly across species**

270 Mature, post-anthesis *Jaltomata* flowers differ substantially in multiple traits, including overall
271 size and relative sizes of individual organs, volume of nectar produced per flower, and trichome
272 type and density on sepals and petals (Table 2; Fig. 1). In terms of floral size, campanulate
273 *Jaltomata calliantha* and *J. dendroidea* have the largest flowers, while rotate *S. pimpinellifolium*
274 has the smallest; *Jaltomata* flowers also differ from *S. pimpinellifolium* in having free stamens
275 (compared to a fused anther cone, considered a derived trait in the wild tomatoes [41]), and in

276 producing nectar. Calyx diameter significantly differs across all species, while both corolla and
277 ovary diameter significantly differ between all species pairs, except tubular *J. umbellata* versus
278 *S. pimpinellifolium* (ANOVA $p < 0.00001$; Tukey HSD, $p < 0.0001$), which both have
279 comparatively small flowers. Although mature floral organ sizes vary substantially among
280 *Jaltomata* species, the ancestrally rotate form of *J. darcyana* flowers have the shortest petals
281 (ANOVA $p < 0.00001$; Tukey HSD, $p < 0.0001$), and the smallest amount of corolla fusion
282 (ANOVA $p < 0.00001$; Tukey HSD, $p < 0.00001$) (Table 2).
283 In addition to organ size and shape, our focal species also differ in the amount of nectar
284 produced per flower (ANOVA $p < 0.00001$; Tukey HSD, $p < 0.0001$) (Table 2), as well as
285 trichome type and density. In particular, the campanulate species *J. calliantha* and *J. dendroidea*
286 as well as tubular *J. umbellata* produce significantly more nectar than either rotate *J. darcyana* or
287 *J. sinuosa*. Finally, mature flowers differ in the types and density of trichomes on mature floral
288 tissues. From simple to complex: *J. darcyana* has sparse simple (uniseriate) trichomes on both
289 sepals and petals, *J. calliantha* dense simple trichomes on both sepals and petals, *J. umbellata*
290 dense simple and dendritic (i.e. branched) trichomes on sepals but only dense simple ones on
291 petals, *J. dendroidea* very dense dendritic trichomes on both sepals and petals, *J. sinuosa* very
292 dense simple, dendritic and viscous glandular trichomes on sepals and dense simple and viscous
293 glandular ones on petals, while *S. pimpinellifolium* has dense simple and non-viscous (i.e. non-
294 secreting) glandular trichomes on both sepals and petals.
295

296 **The early sequence of floral development is similar across *Jaltomata* species**

297 As expected based on their recent common origin (diverged <5 mya, [38]), the five focal species
298 of *Jaltomata* share a common floral ground plan with each other as well as with the *Solanum*

outgroup *S. pimpinellifolium* (from which they diverged ~15-20 mya, [38]). All six species have very similar development throughout organ initiation, which corresponds to growth up to ~1 mm bud diameter (stages A-F; Table 1). In all species, sepals are the first floral organs to emerge from the ~0.15 mm diameter floral meristem, and do so in an asymmetric manner from the abaxial, to adaxial, to lateral sides (Figs. 2a, 3a, 5a-b, 6a, 7a). Once sepals within the outer whorl (within a flower) are qualitatively of similar size and trichome initiation is apparent, the former elongate from the base to form a partially fused structure (Figs. 2b, 3b, 4a, 5c, 6b, 7a-b). By ~0.25-0.3 mm floral diameter, petals and stamens emerge almost simultaneously, but slightly asymmetrically (Figs. 2c, 3c, 4b-c, 5d-e, 6c-d, 7b), followed by gynoecial initiation by ~0.5 mm diameter (Figs. 2f, 7c). Similar to sepals, petals initially emerge as free strap-like primordia, but begin to elongate from the congenitally fused region at the base (just above the stamen attachment point) by 0.7-0.8 mm diameter in all species (Figs. 2e, 3d, 4e, 5f-g, 6g, 7d). Simple (uniseriate) trichomes in *Jaltomata* species, and both simple and non-viscous glandular ones in *S. pimpinellifolium*, are first apparent on petals by ~0.5 mm buds, followed by the emergence of dendritic petal trichomes in *J. dendroidea* and viscous glandular ones in *J. sinuosa* by 1 mm buds (Figs. 2e, 3d-e, 4d-e, 5g-h, 6f, 7e). Finally, the gynoecium is fused, and style elongation started, by ~1 mm bud diameter in all species (Table 1; Figs. 2g, 3f, 4g-h, 5h-i, 6h-i). Because floral organs initiate at the same developmental age across species, observed differences in mature flowers are therefore not a result of differences in growth onset.

Differential bud growth among species is apparent following organ initiation

Following the completion of floral organ initiation in ~1 mm diameter buds, whole buds and their constituent organs continue to grow (including from the congenitally fused base for sepals

322 and petals), anthers begin to mature with the appearance of two lobes or thecae, and trichomes
323 mature on sepals and petals ("stage G" in Table 1). However, also during this stage, conspicuous
324 differences in overall bud size as well as particular floral organs become apparent among
325 species. Indeed, bud diameter begins to significantly differ among species during this mid-stage
326 of floral development (ANOVA $p < 0.0001$), with *S. pimpinellifolium* buds remaining
327 significantly smaller than those of all *Jaltomata* species except *J. umbellata* (Tukey HSD, $p =$
328 0.26 against *J. umbellata*, and $p < 0.001$ for all others), and *J. umbellata* remaining significantly
329 smaller than all other *Jaltomata* species except *J. dendroidea* (Tukey HSD, $p = 0.45$ against *J.*
330 *dendroidea*, and $p < 0.05$ for all others; Table 1). Despite this, corolla traits (corolla depth,
331 corolla fusion, and petal length) do not significantly differ among species (ANOVA, all $p > 0.1$;
332 Table S2), suggesting that their growth rates may be elevated in relation to whole bud growth
333 within *S. pimpinellifolium* and *J. umbellata* (see below).

334 In addition to differences in bud and organ size among species, differences in petal
335 trichome distribution also begin to emerge during stage G. In particular, trichomes along petal
336 lobe edges (i.e. the portion of petals that are not part of the congenitally fused corolla tube)
337 become interlocked, within *Jaltomata* species. Such interlocking trichomes extend from the
338 region of congenitally fused corolla tube to petal tips, and appear to hold the edges of petal lobes
339 together. We refer to this as 'superficial -' or 'functional fusion', as it does not result from fused
340 petal tissue and petal lobes are easily separated once trichomes are pulled apart (Figs. 2h-k, 3f, h-
341 i, 4g, i, 5h, 6g-h). This 'superficial fusion' is most apparent in buds of rotate *J. darcyana* and *J.*
342 *sinuosa*, as well as campanulate *J. calliantha* and *J. dendroidea*, and only to a lesser extent
343 within tubular *J. umbellata*. In particular, in rotate corollas of *J. darcyana* and *J. sinuosa*, the
344 proportion of the congenitally fused corolla tube to region of 'superficial fusion' via interlocking

trichomes remains similar throughout mid-stages; while in campanulate corollas of *J. calliantha* and *J. dendroidea* the congenitally fused region becomes proportionately larger during this time. In contrast to *Jaltomata*, petals in *S. pimpinellifolium* remain only slightly congenitally fused at the base, with no 'superficial fusion' via interlocking trichomes on lobe edges, during this stage (Fig. 7h).

During late stages of floral development, fully differentiated organs (especially petals) continue to grow and mature (stages H-I, Table 1) until petals open at anthesis (stage J, Table 1). Differences in bud diameter are especially pronounced during these late stages, with all species pairs significantly differing (ANOVA, $p < 0.0001$), except *J. umbellata* and S. *pimpinellifolium* (Tukey HSD, $p = 0.99$) during stage H, and *J. calliantha* and *J. dendroidea* (Tukey HSD, $p = 0.47$) and *J. umbellata* and S. *pimpinellifolium* (Tukey HSD, $p = 0.99$) during stage I (Table 1). At anthesis, trichomes providing 'superficial fusion' become unlocked, releasing petal lobes, while congenitally fused regions of the corolla remained fused in mature flowers.

Nectar secretion within *Jaltomata* flowers also begins during stages H-I of floral development, although there are some differences in timing among species. In *J. darcyana*, minute amounts of nectar are apparent in mid-day -1 day buds (i.e. the afternoon of the day before anthesis, during stage I); while for *J. calliantha*, *J. dendroidea*, and *J. sinuosa,* nectar is first apparent during the morning in 0 day buds (i.e. the morning of the day the flower opens, typically a few hours prior to full anthesis, during the very end of stage I); and in *J. umbellata*, nectar secretion is not apparent until the evening of day 0 flowers (after anthesis has occurred, during stage J), or until the morning of +1 day flowers (i.e. the day following anthesis, just prior to anther dehiscence in this species).

368 **Overall duration of floral development and absolute growth rates differ among species**

369 We measured the duration of floral development in days to assess evidence that an extended
370 growth period underpinned species floral differences (i.e. whether larger flowers grow for a
371 longer period), as well as to calculate an average overall growth rate (bud diameter in mm per
372 day) to assess potential accelerated growth of whole flowers.

373 The duration of floral development from 1 mm diameter buds onwards, which
374 corresponds to completion of organ initiation in all species (i.e. starting in stage G, Table 1), was
375 significantly different among species (Table 3). Floral development duration ranged from 14
376 days in outgroup *S. pimpinellifolium* to an average of 32.40 days in *J. dendroidea*. Among
377 *Jaltomata* species, the ancestrally rotate flowers of *J. darcyana* (average 19.23 days) and derived
378 tubular flowers of *J. umbellata* (average 18.62 days) had the shortest development times
379 (ANOVA $p < 0.00001$; Tukey HSD against all other species, $p < 0.00001$; Tukey HSD against
380 each other, $p = 0.525$). Similarly, overall average bud growth rate (bud diameter in mm per day)
381 ranged from 0.185 mm/day in *J. umbellata* to 0.473 mm/day in campanulate *J. dendroidea*
382 (Table 3).

383 Because we also measured bud diameter while assessing duration of floral development,
384 we compared these data with our measures from destructively sampled buds at different sizes to
385 approximate how long (in days) each species remained at the different developmental stages
386 (comprising mid- and late stages, Table 1). While this provides an imperfect estimate of the
387 developmental time-course, it allowed us to assess whether there are evident qualitative
388 differences between species. Based on this comparison, all *Jaltomata* species remained in stage F
389 for approximately 2 days, but spent different amounts of time in mid- and each of the late stages
390 (stages G-I) (Table 1). Rotate *J. darcyana* and tubular *J. umbellata* both spent fewer days in

stage G than the other species. Interestingly, rotate *J. sinuosa* and campanulate *J. calliantha* followed similar time-courses, while campanulate *J. dendroidea* likewise had a similar time-course with the exception of a greatly extended duration of stage I.

Relative floral organ growth rates differ among species

Given observed allometric differences in organ sizes among species (Table 2), we also calculated relative growth rates of individual floral organs. For instance, even if species do not differ in their overall absolute growth rate (diameter per day; Table 3), differences in the relative growth rates among floral organs (floral organ in mm per mm of bud diameter) could explain observed differences in mature flowers. Because we determined that overall bud growth rates do not differ among species until after completion of organ initiation (Table 1), we assessed relative organ growth rates during early stages separately from mid- and late stages (as outlined in Table 1).

We detected several differences among species in relative growth rates for particular floral organs, in both the early and the mid to late stage comparisons (Table 4, Fig. 8). First, during early stages, growth rates of all organs except the ovary and style are significantly elevated in rotate *S. pimpinellifolium* compared to rotate *J. darcyana* (all $p < 0.00001$, except $p = 0.18$ for ovary and $p = 0.75$ for style). Within *Jaltomata*, the growth rate for sepals is significantly elevated in campanulate *J. calliantha*, corolla fusion is significantly decreased in campanulate *J. dendroidea*, and as suggested by organ comparisons by stage (above), corolla depth is significantly elevated in tubular *J. umbellata* (Table 4). Comparing growth rates between campanulate *J. calliantha* and *J. dendroidea*, all corolla traits except fusion are significantly decreased in *J. dendroidea* (Table 4). Against *S. pimpinellifolium*, most organ growth rates are significantly decreased in *Jaltomata* species (indicative of general accelerated

growth in *S. pimpinellifolium* starting in early stages), while only corolla growth rates are significantly decreased in *J. dendroidea* against both *J. darcyana* and *S. pimpinellifolium* (Table S3).

Within mid- and late stages of floral development, we detected even more differences in organ growth rates (Table 4, Fig. 8). Focusing on corolla growth within *Jaltomata*, corolla depth continues to be significantly elevated in *J. umbellata*; corolla fusion and petal length are significantly elevated in all other species; and lobe length is significantly decreased in *J. calliantha* (Table 4). In general, growth rates for corolla depth, corolla fusion, and petal length are highest in tubular *J. umbellata*. Growth rates for corolla fusion and petal length are similar between campanulate species (*J. calliantha* and *J. dendroidea*) and rotate *J. sinuosa*, however corolla depth and lobe growth rates are significantly elevated in *J. dendroidea* compared to *J. calliantha* (Table 4). Comparing against *S. pimpinellifolium*, corolla fusion is elevated and lobe length is decreased in rotate and campanulate *Jaltomata* species, and all corolla traits are elevated in tubular *J. umbellata*; while against both *J. darcyana* and *S. pimpinellifolium* all corolla traits are elevated in the other *Jaltomata* species, except lobe growth in *J. calliantha* (Table S3).

Discussion

Heterochronic shifts during development are often considered a primary mechanism underlying phenotypic evolution [6]. Indeed, heterochrony appears to be a common mechanism underlying morphological diversification in numerous animal lineages [12], as well as in flowers, including shifts in both duration of floral development and growth rates leading to phenotypic shifts in descendent lineages [3-4, 17]. In this study, we examined floral ontogeny in five florally diverse

species of *Jaltomata*, as well as a closely related outgroup that shares the ancestral rotate corolla form, to assess evidence for heterochrony contributing to observed variation in mature corolla forms and other floral traits. Overall, we found that the sequence of developmental events is consistent among examined species, but that differences in growth rate and duration of floral development are associated with different corolla morphologies. In particular, we found that early floral development (<1 mm bud diameter, corresponding to organ initiation) is very similar among all examined species, that differences between *Jaltomata* and *Solanum* are apparent soon after organ initiation (i.e. starting in stage G, Table 1), and that corolla trait differences associated with divergent corolla shapes among *Jaltomata* species first arise during mid-developmental stages. Elevated growth rates of corolla traits, combined with extended duration of floral development, lead to observed differences in mature floral traits among species. In particular, these developmental changes are heterochronic, with predominately accelerated growth rates and extended development duration (that is, peramorphic changes) associated with variation in mature corolla form.

Extended duration of floral development and accelerated growth rates underlie derived tubular and campanulate mature corolla shapes in *Jaltomata*

One goal of this study was to assess whether derived tubular and campanulate corolla forms in *Jaltomata* arise due to heterochronic developmental shifts. In particular, we hypothesized that derived campanulate and tubular forms are elaborated versions of the ancestral rotate form, and therefore might result from peramorphic changes (i.e. longer duration of floral development or accelerated growth rates). Indeed, compared to ancestrally rotate flowers of *J. darcyana* (or indeed, more generally to *S. pimpinellifolium* or a composite of these two species), all derived

460 *Jaltomata* species show peramorphic changes during floral development. First, all species except
461 *J. umbellata* have significantly longer development duration (Table 3). Because floral organs
462 initiated at the same developmental age across species, differences in the duration of floral
463 development in derived forms result from changes in the timing of offset (i.e. growth period is
464 extended). Second, growth rates for most of our measured floral traits are significantly elevated
465 in *J. umbellata* compared to *J. darcyana*, including petal length and extent of corolla fusion
466 (Table 4), even though *J. darcyana* and *J. umbellata* have similar development periods (19.23
467 days vs. 18.62 days, Table 3). Similarly, growth rates of most floral organs are significantly
468 elevated in both campanulate species – including rates of petal length and extent of corolla
469 fusion – in comparison to *J. darcyana* (Table 4), even though both also have longer development
470 periods and faster overall growth rates in terms of bud diameter (Table 2). Thus, derived
471 campanulate flowers in *J. calliantha* and *J. dendroidea* result from both accelerated organ
472 growth rates and an extended development period, while derived tubular flowers in *J. umbellata*
473 appear to result from accelerated growth rates of particular floral organs and organ components.
474 These findings are consistent with other comparative ontogenetic studies examining floral
475 morphology transitions associated with pollinator shifts, in which the derived floral form appears
476 to be an elaborated version of the ancestral (e.g. [33]). For instance, peramorphic growth was
477 found to underlie the elaborated nectariferous petal in the derived hummingbird pollinated
478 *Delphinium* [33]. In contrast, in cases where the derived form appears to be a more juvenilized
479 version, pedomorphic shifts predominantly explain these differences [4, 34-35].

480 In addition to changes associated with morphological differences between species, we
481 also determined that nectar secretion dynamics appear to have undergone a combination of
482 heterochronic shifts in more derived forms, including a delayed onset of the start of nectar

483 secretion, as well as increased rates of nectar production. However, these shifts were found in
484 rotate *J. sinuosa*, both campanulate *J. calliantha* and *J. dendroidea*, as well as tubular *J.*
485 *umbellata*, suggesting that variation in nectar production is genetically and/or developmentally
486 uncoupled from corolla morphology. Indeed, preliminary quantitative trait locus (QTL) mapping
487 in a hybrid population generated from *J. sinuosa* and *J. umbellata* (Kostyun et al., unpublished)
488 indicates that QTL for aspects of corolla morphology and nectar volume do not coincide. This is
489 in contrast to studies in several other systems (reviewed in [48]) in which overlapping QTL were
490 identified for floral shape and reward characters, although these cases as yet are unable to
491 distinguish closely linked, but distinct, loci for corolla morphology and nectar traits, from
492 actually pleiotropic loci.
493

494 **Alike mature corolla forms result from similar heterochronic changes during mid-stage**
495 **ontogeny**

496 Another goal of this study was to assess whether alike mature corolla shapes arise from shared or
497 divergent developmental trajectories. Overall, we found that floral development was very similar
498 during early and mid-stages within both species with rotate flowers, suggesting that these corolla
499 morphologies may share a single evolutionary origin (i.e. are homologous). In contrast, species
500 with campanulate flowers show several different types of changes, albeit in similar directions,
501 supporting our hypothesis that the campanulate corolla morphologies in these two species are
502 independently derived.

503 Floral ontogeny in general, and corolla development in particular, are very similar
504 between rotate flowers of *J. sinuosa* and ancestrally rotate flowers of *J. darcyana*, until late
505 stages when growth of petal length and corolla fusion become significantly elevated in *J. sinuosa*

506 (Table 4). These species also share a similar overall bud growth rate, although *J. sinuosa*
507 continues to grow for significantly longer (Table 3). These data indicate that the shared rotate
508 floral morphology in these two species arises through a very similar developmental process, but
509 peramorphic changes (both an extended development period and growth acceleration of
510 particular floral organs) during late floral development lead to an overall increased size of *J.*
511 *sinuosa* flowers, and a proportionately greater extent of corolla fusion (Table 2). These
512 observations again suggest that the rotate flowers in these species share a single evolutionary
513 origin.

514 In contrast, we determined that while both campanulate species have elevated corolla
515 fusion and petal growth starting in mid-stage ontogeny (Table 4), they also significantly differ in
516 perianth (sepal and petal) growth during early stages (Table 4), as well as in the duration of floral
517 development (Table 3) and estimated duration of different stages (Table 1). Additionally, *J.*
518 *calliantha* actually shows a significantly decreased growth rate (i.e. neoteny) for lobe length
519 compared to *J. darcyana* during mid- and late stages (Table 4), whereas the growth rate of lobe
520 length does not significantly differ between *J. dendroidea* and *J. darcyana*. These differences
521 suggest that campanulate corollas in these two species are independently derived. Alternatively,
522 these forms could share a single origin, but could have experienced subsequent developmental
523 changes without large changes in the mature phenotype (i.e. developmental systems drift) during
524 lineage divergence. In the absence of other data, we prefer the former hypothesis based on the
525 phylogenetic distribution of these two species [37]; (Wu, Kostyun, Moyle, unpublished) (Fig. 1).
526 Together, these observations agree with prior studies suggesting that different heterochronic
527 changes contributing to similar mature floral phenotypes may actually be quite common; for
528 instance, multiple distinct developmental shifts have been shown to produce flowers with the

529 ‘selfing syndrome’ across lineages [3-4, 23] and among closely related populations and species
530 [24].
531

532 **Heterochronic shifts as a first step to identifying specific mechanisms underlying floral**
533 **divergence and convergence in *Jaltomata***

534 Our data suggest an important role for several different heterochronic developmental shifts in
535 generating floral trait diversity among *Jaltomata* species, especially peramorphism (via growth
536 acceleration and extended growth period), in shaping corolla diversity. If these heterochronic
537 shifts are caused by relatively simple genetic changes, this could have contributed to the apparent
538 rapid floral trait diversification within the genus (<5 my; [38]). Future work identifying the
539 specific genetic and developmental mechanisms underlying these heterochronic shifts will
540 provide a better understanding of the pace of phenotypic change, as well as likely candidate
541 genes contributing to these developmental shifts. For instance, changes in the regulation of cell
542 proliferation and/or cell expansion can lead to variation in overall floral size or size of particular
543 floral organs, and several key candidate genes have been identified in *Arabidopsis* that function
544 during these processes [23, 49]. Given our observed patterns of developmental heterochrony (i.e.
545 extended floral development duration and accelerated growth), we anticipate that a combination
546 of molecular heterochrony (e.g. growth promoting factors are expressed for longer) and
547 heterometry (e.g. a higher amount of growth promoting gene products are produced) might
548 underlie these shifts.

549 In addition to intrinsic (genetic) regulation of cell growth within floral organs, external
550 signals or structures could also influence mature floral morphology [50-51]. For instance, the
551 involvement of trichomes in regulating flower shape was recently reported in cotton (*Gossypium*

552 *hirsutum*), in which the petal trichome gene *GhMYB-MIXTA-Like10* (*GhMYBML10*) is strongly
553 expressed at the point of petal overlap, resulting in trichome cross-linking that physically hold
554 petals in place [46]. We identified similar 'superficial corolla fusion' via interlocking trichomes
555 in both rotate and campanulate *Jaltomata* species examined here, although different growth
556 dynamics during mid- and late-stages was observed between these forms. Although trichome
557 cross-linking occurs in these species, it remains to be determined whether it is essential for
558 normal corolla development (as in [46]) and if this phenotype is regulated by a *MYBML10*
559 orthologue (including whether expression differs between rotate and campanulate vs. tubular
560 *Jaltomata* forms).

561 The identification of specific developmental mechanisms and candidate genes will also
562 clarify whether similar mature floral morphs result from shared evolutionary history or from
563 convergent or parallel changes at the molecular level. In the *Jaltomata* species that we examined
564 here, floral development (especially for corolla traits) in both rotate species was extremely
565 similar until late stages, while in the campanulate species, we identified heterochronic shifts in
566 the same direction but not in the same characters. Thus, it is likely that *J. darcyana* and *J.*
567 *sinuosa* share their rotate form from common ancestry, while *J. calliantha* and *J. dendroidea*
568 represent convergent or parallel evolution of the campanulate form. In this way, determining the
569 developmental mechanisms underlying these heterochronic shifts can be used both to pinpoint
570 how changes in existing floral development pathways lead to phenotypic variation, and to
571 differentiate alternative evolutionary histories (common ancestry versus convergence) for similar
572 mature floral forms. In particular, shared mutations would be more indicative of common
573 descent rather than parallel evolution.
574

575 **Pollinator-mediated selection may have shaped *Jaltomata* floral diversification**

576 Understanding how developmental trajectories differ between species with different mature
577 floral traits also broadly informs the ways in which these traits are most able to respond to
578 selection (e.g. whether developmental constraint restricts certain paths of trait evolution). Such
579 inferences are especially relevant to floral trait evolution across the *Jaltomata* genus, which
580 shows both high levels of floral trait divergence, as well as multiple putatively independent shifts
581 to similar floral forms, within a relatively short timescale (<5mya, [38]). From an evolutionary
582 perspective, such shifts are likely to have been shaped by pollinator behavior. Observations
583 indicate that some species are predominantly visited by distinct pollinator functional groups (e.g.
584 hymenopterans vs. hummingbirds, T. Mione, pers. comm.; J.L. Kostyun, unpublished), and these
585 species have floral trait suites consistent with different pollinator syndromes [52]. In particular,
586 hummingbirds have been observed visiting campanulate *J. calliantha* whose flowers have
587 copious amounts of dilute nectar [53] as well as and tubular *Jaltomata viridiflora*, while
588 hymenopterans have been observed visiting *J. sinuosa* and *Jaltomata repandidentata* (a close
589 relative to *J. darcyana*; [37]) that have rotate corollas with relatively small amounts of
590 concentrated nectar [54]. Because differential pollinator behavior often leads to reproductive
591 isolation between lineages [55], understanding the underlying developmental basis of floral trait
592 evolution can also reveal factors that could accelerate speciation in this rapidly-evolving and
593 florally diverse system.

594

595 **Conclusions**

596 As articulated by Darwin, vast diversity of form (including in flowers) can arise from changes in
597 late developmental stages despite early developmental similarities that are rooted in common

598 ancestry. One such type of developmental change is heterochrony, which is a shift in rate or
599 timing in a descendant compared to its ancestor. By comparing floral ontogeny among five
600 *Jaltomata* species and an outgroup, we determined that heterochronic shifts during mid- and late
601 stages of floral development distinguish divergent corolla forms. In particular, two types of
602 peramorphosis (differential growth acceleration and delayed offset leading to an extended
603 development period) predominately explain these changes. Our data therefore support Darwin's
604 insight that even highly divergent mature floral traits result from modifications to initially similar
605 structures. These relatively simple heterochronic shifts contribute to observed floral trait
606 variation among *Jaltomata* species and, as such, could act as a mechanism allowing rapid floral
607 trait diversification in this florally diverse system.
608

609 **Declarations**

610 **Ethics approval and consent to participate**

611 Not applicable.
612

613 **Consent for publication**

614 Not applicable.
615

616 **Availability of data and material**

617 Summaries of datasets used and/or analyzed during the current study are available within the
618 Supplementary Materials, while raw data is available on Dryad [DOI to be added upon
619 acceptance]. Seed material was obtained from Dr. Thomas Mione at Central Connecticut State

University and the Tomato Genetics Resource Center at UC Davis – both of which followed all applicable regulations in obtaining the material.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by a National Science Foundation Graduate Research Fellowship (NSF DEB 1342962) and Amherst College Graduate Fellowship to JLK, a National Science Foundation Award (NSF IOS 1353056) to JCP, and a National Science Foundation Award (NSF DEB 1136707) to LCM. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

Authors' contributions

All authors planned and designed the research, JLK performed the experiments, JLK and JCP analyzed the data, and all wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We thank Armin Moczek and three anonymous reviewers for providing feedback on a previous draft of this manuscript, Barry Stein of the IU Electron Microscopy Center and Michele von

642 Turkovich of the UVM Microscopy Imaging Center for SEM and other instrument training, the
643 IU Greenhouse staff for plant care, and Thomas Mione for sharing seed material.

644

645 **References**

646 1. Darwin C. On the Origin of species by means of natural selection, or, the preservation of
647 favoured races in the struggle for life. London: J. Murray; 1859.
648 2. Friedman WE, Diggle PK. Charles Darwin and the origins of plant evolutionary
649 developmental biology. Plant Cell 2011; 23:1194-1207.
650 3. Li P, Johnston MO. Heterochrony in plant evolutionary studies through the twentieth century.
651 Bot Rev 2000; 66:57-88.
652 4. Box MS, Glover BJ. A plant developmentalist's guide to paedomorphosis: reintroducing a
653 classic concept to a new generation. Trends Plant Sci 2010; 15:241-246.
654 5. Haeckel E. Die Gastrula and die Eifurchung der Thiere. Jena Z. Naturwiss 1875; 9:402-508.
655 6. de Beer GR. Embryology and Evolution. Oxford: Clarendon Press; 1930.
656 7. de Beer GR. Embryos and ancestors. Oxford: Clarendon Press; 1940.
657 8. Gould SJ. Ontogeny and phylogeny. Cambridge, MA: Harvard University Press; 1977.
658 9. Gould SJ. Ontogeny and phylogeny: revisited and reunited. BioEssays 1992; 14:275-279.
659 10. Klingenberg, CP. Heterochrony and allometry: The analysis of evolutionary change in
660 ontogeny. Biol Rev 1998; 73:79-123.
661 11. Smith KK. Sequence heterochrony and the evolution of development. J Morphol 2002;
662 252:82-97.
663 12. McNamara KJ. Heterochrony: the evolution of development. Evo Edu Outreach 2012; 5:203-
664 218.

665 13. Wayne RK. Cranial morphology of domestic and wild canids: the influence of development
666 on morphological change. Evolution 1986; 40:243-261.
667 14. Truman JW, Riddiford LM. The origins of insect metamorphosis. Nature 1999; 401:447-452.
668 15. Cartolano M, Pieper B, Lempe J, Tattersall A, Huijser P, Tresch A, Darrah PR, Hay A,
669 Tsiantis M. Heterochrony underpins natural variation in *Cardamine hirsuta* leaf form.
670 Proc Natl Acad Sci USA 2015; 112:10539-10544.
671 16. Lemmon ZH, Park SJ, Jiang K, Van Eck J, Schatz MC, Lippman ZB. The evolution of
672 inflorescence diversity in the nightshades and heterochrony during meristem maturation.
673 Genome Res 2016; 26:1676-1686.
674 17. Geuten K, Coenen H. Heterochronic genes in plant evolution and development. Front Plant
675 Sci 2013; 4:381.
676 18. Endress PK. Evolutionary diversification of the flowers in angiosperms. Am J Bot 2011;
677 98:370-396.
678 19. Angiosperm Phylogeny Group, The. An update of the Angiosperm Phylogeny Group
679 classification for the orders and families of flowering plants: APG IV. Bot J Linn Soc
680 2016; 181:1-20.
681 20. Stebbins GL. Self fertilization and population variability in the higher plants. Am Nat 1957;
682 91:337-354.
683 21. Stebbins GL. Adaptive radiation of reproductive characteristics in angiosperms, I: Pollination
684 mechanisms. Annu Rev Ecol Evol Syst 1970; 1:307-326.
685 22. Barrett SCH, Harder LD. Floral variation in *Eichhornia paniculata* (Spreng) Solms
686 (Pontederiaceae). 2. Effects of development and environment on the formation of selfing
687 flowers. J Evol Biol 1992; 5:83-107.

688 23. Sicard A, Lenhard M. The selfing syndrome: a model for studying the genetic and
689 evolutionary basis of morphological adaptation in plants. Ann Bot 2011; 107:1433-1443.
690 24. Summers HE, SM Hartwick, Raguso RA. Geographic variation in floral allometry suggests
691 repeated transitions between selfing and outcrossing in a mixed mating plant. Am J Bot
692 2015; 102:745-757.
693 25. Li P, Johnston MO. Comparative floral morphometrics of distyly and homostyly in three
694 evolutionary lineages of *Amsinckia* (Boraginaceae). Canadian Journal of Botany 2001;
695 79:1332-1348.
696 26. Georgiady MS, Lord EM. Evolution of the inbred flower form in the currant tomato,
697 *Lycopersicon pimpinellifolium*. Int J Plant Sci 2002; 163:531-541.
698 27. Runions CJ, Geber MA. Evolution of the self-pollinating flower in *Clarkia xantiana*
699 (Onagraceae). I. Size and development of floral organs. Am J Bot 2000; 87:1439-1451.
700 28. Li P, Johnston MO. Flower development and the evolution of self-fertilization in *Amsinckia*:
701 The role of heterochrony. Evol Biol 2010; 37:143-168.
702 29. Lord EM, Hill JP. Evidence for heterochrony in the evolution of plant form. In: Raff RA,
703 Raff EC, editors. Development as an evolutionary process. New York: Alan R. Liss;
704 1987. p. 47–70.
705 30. Porras R, Munoz, M. Cleistogamous capitulum in *Centaurea melitensis* (Asteraceae):
706 heterochronic origin. Am J Bot 2000; 87:925-933.
707 31. Minter TC, Lord EM. A comparison of cleistogamous and chasmogamous floral
708 development in *Collomia grandiflora* Dougl ex Lindl (Polemoniaceae). Am J Bot 1983;
709 70:1499-1508.

710 32. Luo Y, Bian FH,Luo YB. 2012. Different patterns of floral ontogeny in dimorphic flowers of
711 *Pseudostellaria heterophylla* (Caryophyllaceae). Int J Plant Sci 2012; 173:150-160.
712 33. Guerrant EO. Neotenic evolution of *Delphinium nudicaule* (Ranunculaceae) - a
713 hummingbird-pollinated larkspur. Evolution 1982; 36:699-712.
714 34. Box MS, RM Bateman, BJ Glover, Rudall PJ. Floral ontogenetic evidence of repeated
715 speciation via paedomorphosis in subtribe Orchidinae (Orchidaceae). 2008; Bot J Linn
716 Soc 157:429-454.
717 35. Armbruster WS, J Lee, ME Edwards, Baldwin BG. Floral paedomorphy leads to secondary
718 specialization in pollination of Madagascar *Dalechampia* (Euphorbiaceae). Evolution
719 2013; 67:1196-1203.
720 36. Olmstead RG, L Bohs, HA Migid, E Santiago-Valentin, VF Garcia, Collier SM. A molecular
721 phylogeny of the Solanaceae. Taxon 2008; 57:1159-1181.
722 37. Miller RJ, T Mione, HL Phan, Olmstead RG. Color by numbers: nuclear gene phylogeny of
723 *Jaltomata* (Solanaceae), sister genus to *Solanum*, supports three clades differing in fruit
724 color. Syst Botany 2011; 36:153-162.
725 38. Sarkinen T, L Bohs, RG Olmstead, Knapp S. A phylogenetic framework for evolutionary
726 study of the nightshades (Solanaceae): a dated 1000-tip tree. BMC Evol Biol 2013;
727 13:214.
728 39. Mione T, Leiva González S, Yacher L. Two new Peruvian species of *Jaltomata* (Solanaceae,
729 Solaneae) with red floral nectar. Brittonia 2015; 67: 105-112.
730 40. Hansen DM, JM Olesen, T Mione, SD Johnson, Muller CB. Coloured nectar: distribution,
731 ecology, and evolution of an enigmatic floral trait. Biological Reviews 2007; 82:83-111.

732 41. Knapp S. On 'various contrivances': pollination, phylogeny and flower form in the
733 Solanaceae. Phil Trans R Soc B 2010; 365:449-460.
734 42. Kostyun JL, Moyle LC. Multiple strong postmating and intrinsic postzygotic reproductive
735 barriers isolate florally diverse species of *Jaltomata* (Solanaceae). Evolution 2017;
736 71:1556-1571.
737 43. True JR, Haag ES. Developmental system drift and flexibility in evolutionary trajectories.
738 Evol Dev 2001; 3:109-119.
739 44. Xiao H, C Radovich, N Welty, J Hsu, DM Li, T Meulia, van der Knaap E. Integration of
740 tomato reproductive developmental landmarks and expression profiles, and the effect of
741 SUN on fruit shape. BMC Plant Biol 2009; 9:49.
742 45. Schneider CA, WS Rasband, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis.
743 Nature Methods 2012; 9:671-675.
744 46. Tan J, Walford S-A, Dennis ES, Llewellyn D. Trichomes control flower bud shape by linking
745 together young petals. Nature Plants 2016; 2:16093.
746 47. R Core Team. R: A language and environment for statistical computing. R Foundation for
747 Statistical Computing, Vienna, Austria. 2015. https://www.R-project.org/.
748 48. Smith SD. Pleiotropy and the evolution of floral integration. New Phytol 2016; 209:80-85.
749 49. Krizek BA, Anderson JT. Control of flower size. J Exp Bot 2013; 64:1427-1437.
750 50. Gilbert SF. Ecological developmental biology: environmental signals for normal animal
751 development. Evol Dev 2012; 14:20-28.
752 51. Sampathkumar A, Yan A, Krupinski P, Meyerowitz EM. Physical forces regulate plant
753 development and morphogenesis. Curr Biol 2014; 24:R475-R483.

754 52. Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD. Pollination syndromes
755 and floral specialization. Annu Rev Ecol Evol Syst 2004; 35:375-403.
756 53. Plourd KC, Mione T. 2016. Pollination does not affect floral nectar production, and is
757 required for fruit-set by a hummingbird-visited Andean plant species.
758 Phytologia 2016; 98: 313-317.
759 54. Mione T, Plourd KC, Leiva González S, Yacher L, Philbrick CT, Blume D. Pollination
760 decreases longevity of the protogynous flowers of *Jaltomata sinuosa* (Solanaceae).
761 Journal of Biology and Nature 2017; 7: 123-129.
762 55. Kay KM, Sargent RD. The role of animal pollination in plant speciation: integrating ecology,
763 geography, and genetics. Annu Rev Ecol Evol Syst 2009; 40:637-656.
764 56. Leiva González S, Mione T, Yacher L, Silvestre VQ. *Modillonia* una nueva sección de
765 *Jaltomata* Schlechtendal (Solanaceae) con una nueva especies del Norte del Perú.
766 Arnaldoa 2010; 17: 163-171.
767
768
769
770
771
772
773
774
775
776

Figure legends

Fig. 1. Simplified phylogenetic relationships in *Jaltomata* and the outgroup *Solanum pimpinellifolium* based on [38, 41] and (Wu, Kostyun, Moyle, unpublished). Representative mature flowers are depicted for the species examined here (in bold font). White scale bars = 1 cm. R, rotate; C, campanulate; T, tubular. Note: All five focal *Jaltomata* species are represented in [37], but as this study was accepted prior to formal naming of *J. calliantha* [56], this species is called "J. hummingbird".

Fig 2. *Jaltomata darcyana* rotate flower development. (a,b) Stages A-C: Sepals develop asymmetrically from the abaxial side, and once of similar lengths, grow from a their base to form a fused tube. (c) Stage C: Removal of sepals reveals development of petal and stamen primordia. (d,e) Stage D: Petals grow freely but begin to develop trichomes. (f,g) Stages E-F: Carpels become fused during gynoecial development. (h-k) Stages F-G: Petals are congenitally fused at the base just above the stamen insertion point (h-i), but then become 'superficially fused' via interlocking trichomes along their lengths by ~3 mm flower diameter (j-k). Scale bars = 100 um, and bud diameter is provided in bottom right panel. s = sepal, p = petal, st = stamen, tri = trichome, fus = corolla fusion, fil = anther filament, g = gynoecium, sty = style.

Fig 3. *Jaltomata sinuosa* rotate flower development. (a,b) Stages A-B: Sepals initiate in an asymmetric fashion from the abaxial side, and quickly fuse at the base. (c) Stage C: Five free petals develop around initiating stamen primordia. (d-e) Stage E: As stamens continue to mature,

800 petals remain mostly free, but are fused at the base. (f-g) Stages F-G: Removal of mostly free
801 petals reveals development of fused carpels. (h,i) Stage G: Opening of the corolla in 2.5-4 mm
802 wide flowers shows a fused petal base from just above the stamen attachment point to about
803 mid-way up the length of the corolla (i), and 'superficial fusion' of petals above the mid-point,
804 via interlocking trichomes. Scale bars = 100 um, and bud diameter provided in bottom right
805 corner. s = sepal, p = petal, st = stamen, tri = trichome, fus = corolla fusion, fil = anther filament,
806 g = gynoecium, sty = style.
807
808 **Fig 4**. *Jaltomata calliantha* campanulate flower development. (a) Stage A: Free sepals extend
809 through the growth of a basal primordium to form a lower fused tube. (b) Stage C: Opening of
810 partially fused sepals reveals initiation of petal, followed by stamen, primordia. (c) Stage C: Free
811 petals and stamens grow prior to initiation of the carpel primordia. (d-f) Stages D-E: Free petals
812 develop trichomes on the adaxial side, becoming slightly fused just above the stamen attachment
813 point during stamen thecae emergence. (g-h) Stages F-G: Fused carpel development. (i) Stage G:
814 Petals remain only slightly fused above the stamen filament in 3.5 mm wide flowers. Scale bars
815 = 100 um, and bud diameter is also provided in bottom right corner. s = sepal, p = petal, st =
816 stamen, tri = trichome, fus = corolla fusion, fil = anther filament, g = gynoecium, sty = style.
817
818 **Fig 5**. *Jaltomata dendroidea* campanulate flower development. (a,b) Stage A: Sepals develop
819 asymmetrically from the abaxial side. (c) Stage A: Once equal in size, sepals extend through
820 growth of the underlying meristem, giving rise to a fused tube. (d,e) Stage C: Partial removal of
821 sepals reveals development of petals and stamens. (f-h) Stages D-G: Petals are largely free from
822 early development to stamen thecae differentiation in 1.6 mm wide flowers, however, there is a

823 small zone of fusion about the stamen attachment point. (i) Stage F: Carpels are partially fused
824 above the developing ovary. Scale bars = 100 um, and bud diameter also provided in bottom
825 right corner. s = sepal, p = petal, st = stamen, tri = trichome, fus = corolla fusion, fil = anther
826 filament, g = gynoecium, sty = style.
827
828 **Fig 6**. *Jaltomata umbellata* tubular flower development. (a) Stage A: Sepal development occurs
829 asymmetrically, with the first sepal initiating on the abaxial side. (b) Stage B: Once all sepal
830 primordia have expanded, organs become fused at the base to form a tube. (c,d) Stages C:
831 Removal of partially fused sepals reveals the initiation of petal and stamen primordia in an
832 asymmetric progression. (e-f) Stages C-D: Separate petal primordia elongate without fusion. (g-
833 i) Stages E-H: As stamens continue to mature, petals of ~1-2.6 mm diameter flowers become
834 increasingly fused above the stamen attachment point to form a corolla tube. By stage H (i),
835 petals are longer than sepals. Scale bars = 100 um, and bud diameter also provided in bottom
836 right corner. s = sepal, p = petal, st = stamen, tri = trichome, fus = corolla fusion, fil = anther
837 filament, g = gynoecium, sty = style.
838
839 **Fig. 7.** *Solanum pimpinellifolium* rotate flower development. (a) Sepals develop asymmetrically
840 and fuse at their base to form a fused ring. (b,c) Removal of sepals reveals development of petals
841 and stamens, followed by carpel development (c). (d-h) Petals are fused at the base above the
842 stamen attachment point (visible in g) from early development (d), however, most of the petal
843 margin is free. (i) Late carpel development. (j) At the 2 mm flower diameter stage, when anther
844 thecae are well established, petals are mostly free, but remain partially fused at the base. Scale

bars = 100 um, and bud diameter also provided in bottom right corner. s = sepal, p = petal, st = stamen, tri = trichome, fus = corolla fusion, fil = anther filament, g = gynoecium, sty = style.

Fig. 8. Growth trajectories for the focal floral organs and features. For all panels, the x-axis is bud diameter (mm) and the y-axis is the focal organ (mm). Top: growth rates during early stages (bud measurements during 'stages A-F'); and Bottom: growth rates during mid- and late stages (bud measurements during 'stages G-I'). Data points as well as regression lines of best fit are color coded by species. Slopes on log-transformed values are presented in Tables 4 and S3.

Additional files

Additional file 1: Tables S1-S3. Table S1. Accessions of the species used in the present study and their collection locations. All *Jaltomata* seed material provided by Dr. Thomas Mione at Central Connecticut State University (CCSU); *S. pimpinellifolium* seed provided by the Tomato Genetics Resource Center at UC Davis. **Table S2**. Summaries of average floral organ size at different stages of development across species. **Table S3**. Relative growth rates for floral organs for the examined species, measured against bud diameter, for early stages only, mid+late stages only, and overall development. Measurements were log-transformed prior to analysis. Significant differences against baseline indicated by * (* $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$), with black against J. darcyana, red against *S. pimpinellifolium* (alternate ancestral rotate), and blue against *J. darcyana* + *S. pimpinellifolium* ('composite' ancestral rotate); significant differences between *J. calliantha* and *J. dendroidea* (convergent campanulate) indicated by ^ (^ $p < 0.05$; ^^ $p < 0.001$; ^^^ $p < 0.0001$). Additional file 1 is in .xslx format.

868 **Additional file 2: Figure S1.** Measured morphological traits on mature flowers. Floral organs
869 were measured on three flowers per individual, for at least three individuals per species, using
870 hand-held digital calipers to the nearest 0.01 mm. Calyx diameter was measured as the widest
871 planar distance across the calyx (outer most whorl); sepal length from the tip of the sepal to the
872 center of the calyx; corolla diameter as the widest planar distance across the corolla (second
873 whorl); corolla depth as the vertical distance from the base of the corolla to its highest point,
874 petal length from the tip of the petal to the center of the corolla base; corolla fusion from the base
875 of the corolla to the highest point of congenitally fused corolla; petal lobe length from the lowest
876 point of non-fused petal edge to petal tip; stamen length from the base of the stamen (anther
877 filament) to the tip of the anther; ovary diameter as the widest planar distance across the base of
878 the ovary (inner most whorl); and style length from the base of the style to its tip (stigma).
879 Additional file 2 is in .pdf format.

Table 1. Bud development stages based on morphological markers, means (and ranges, in parentheses, for mid- and late stages) of bud diameter for each stage across species, and estimates of duration (in days) spent in each stage.

			J. darcyana			*J. sinuosa*			*J. calliantha*		
			Dia Mean	**S.D.**	**Days^**	**Dia Mean**	**S.D.**	**Days^**	**Dia Mean**	**S.D.**	**Days^**
EARLY	**A**	**Sepals initiated**	0.20 [a]	0.03		0.17 [a]	0.03		0.17 [a]	ND	
	B	**Petals initiated; sepal trichomes initiated**	0.27 [a]	0.03		0.27 [a]	0.04		0.28 [a]	0.06	
	C	**Stamens initiated**	0.39 [a]	0.07		0.36 [a]	0.04		0.37 [a]	0.07	
	D	**Gynoecium initiated; petal trichomes initiated**	0.62 [a]	0.05		0.60 [a]	0.07		0.60 [a]	0.08	
	E	**Stamen filament/anther distinguished; corolla tube apparent**	0.72 [a]	0.08		0.69 [a]	0.09		0.83 [a]	0.07	
	F	**Gynoecium fused; style elongation started**	1.05 [a]	0.16	2	0.86 [a]	0.06	2	0.91 [a]	0.11	2
MID	**G**	**Anther thecae distinguished; petal trichomes mature**	2.93 [a] (1.3 - 4.0)	0.88	14	2.65 [a] (0.9 - 4.8)	1.40	20	2.98 [a] (1.0 - 5.5)	1.46	20
LATE	**H**	**Petals outgrow sepals**	4.50 [a] (4.0 - 4.8)	0.23	2	5.38 [b] (5.0 - 5.7)	0.22	4	7.97 [c] (5.5 - 10.0)	1.27	4
	I	**Sepals open fully**	5.10 [a] (4.8 - 5.4)	0.23	1	6.79 [b] (5.9 - 7.5)	0.53	3	11.22 [c] (10.0 - 12.0)	0.59	3
	J*	**Petals open (anthesis); nectar secretion started in *Jaltomata***	5.00 [a]	0.32		7.16 [b]	0.45		12.13 [c]	0.36	

Significant differences among species are indicated by different letters ($p<0.001$ following Bonferri Correction, with ANOVA and Tukey's HSD). *Note: Bud diameter data are from measurements on destructively sampled buds throughout development, except for stage J which were obtained from the separate experiment measuring duration of floral development (i.e. Table 3). Average bud diameter at stage J are therefore provided here to represent fully mature buds

just prior to anthesis (i.e. the end of floral ontogeny as measured in this study). ^Note: Days refers to an estimate of how many days buds remain at each stage, based on the experiment measuring duration of floral development starting at 1 mm buds.

Table 1 (continued).

J. dendroidea			*J. umbellata*			*S. pimpinellifolium*		
Dia Mean	**S.D.**	**Days^**	**Dia Mean**	**S.D.**	**Days^**	**Dia Mean**	**S.D.**	**Days^**
0.21 [a]	0.04		0.20 [a]	0.01		0.18 [a]	0.03	
0.29 [a]	ND		0.24 [a]	0.02		0.26 [a]	0.02	
0.42 [a]	0.08		0.42 [a]	0.07		0.39 [a]	0.08	
0.64 [a]	0.05		0.59 [a]	0.08		0.53 [a]	0.10	
0.81 [a]	0.11		0.86 [a]	0.24		0.69 [a]	0.07	
1.02 [a]	0.07	2	0.91 [a]	0.07	2	0.80 [a]	0.23	1
2.51 [ab] (1.0 - 5.5)	1.62	20	1.65 [bc] (1.0 - 2.3)	0.40	10	0.96 [c] (0.7 - 1.2)	0.17	4
6.29 [d] (5.5 - 7.0)	0.55	2	2.81 [e] (2.3 - 3.3)	0.27	2	2.77 [e] (2.4 - 3.0)	0.17	2
10.96 [c] (7.0 - 15.5)	2.69	8	3.18 [d] (3.1 - 3.6)	0.26	5	3.12 [d] (3.0 - 3.3)	0.10	4
15.30 [d]	0.27		3.44 [e]	0.11		2.75 [f]	0.19	

Table 2. Mature floral trait means for the five included *Jaltomata* species and *Solanum pimpinellifolium*.

	J. darcyana (n=7)		*J. sinuosa* (n=7)		*J. calliantha* (n=7)		*J. dendroidea* (n=3)		*J. umbellata* (n=5)		*S. pimpinellifolium* (n=3)	
	Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Calyx Diameter (mm)	11.88 [a]	0.37	15.49 [b]	0.40	33.88 [c]	1.21	19.69 [d]	1.74	8.14 [e]	1.63	5.77 [f]	0.43
Sepal Length (mm)	6.32 [a]	0.16	7.48 [b]	0.25	18.01 [c]	1.10	9.67 [d]	0.80	4.41 [e]	0.73	3.45 [f]	0.23
Corolla Diameter (mm)	20.48 [a]	2.18	29.82 [b]	2.27	27.48 [c]	3.48	35.35 [d]	3.27	15.32 [e]	2.52	15.58 [e]	0.58
Corolla Depth (mm)	0.77 [a]	0.09	1.38 [a]	0.27	15.57 [b]	1.84	12.72 [c]	0.88	10.25 [d]	2.27	0.45 [a]	0.07
Corolla Fusion (mm)	3.96 [a]	0.28	9.45 [b]	0.46	15.64 [c]	0.44	14.91 [c]	3.78	10.33 [b]	1.22	1.60 [d]	0.25
Corolla Fusion Proportion	0.42 [a]	0.07	0.64 [b]	0.05	0.86 [c]	0.08	0.65 [b]	0.10	0.72 [b]	0.08	0.21 [d]	0.03
Lobe Length (mm)	5.48 [a]	1.42	5.40 [a]	0.70	2.77 [b]	1.83	7.65 [c]	0.66	4.08 [a]	0.96	5.97 [a]	0.11
Petal Length (mm)	9.53 [a]	1.39	14.85 [b]	1.04	18.41 [c]	1.92	22.56 [d]	2.98	14.40 [b]	0.81	7.57 [e]	0.22
Stamen Length (mm)	4.76 [a]	0.38	10.80 [b]	0.60	10.48 [b]	1.00	10.94 [b]	0.35	10.45 [b]	0.57	6.15 [c]	0.09
Anther Length (mm)	1.43 [a]	0.10	1.59 [b]	0.10	3.27 [c]	0.19	1.86 [d]	0.05	1.34 [a]	0.15	5.75 [e]	0.12
Ovary Diameter (mm)	1.78 [a]	0.14	2.28 [b]	0.19	5.30 [c]	0.41	3.15 [d]	0.18	1.37 [e]	0.26	1.12 [e]	0.06
Style Length (mm)	4.79 [a]	0.53	7.90 [b]	0.73	8.38 [b]	0.66	12.74 [c]	0.86	14.64 [d]	1.47	5.45 [a]	0.21
Nectar Volume (ul)	2.19 [a]	0.48	6.86 [b]	1.31	50.62 [c]	3.76	32.89 [d]	7.03	17.80 [e]	3.84	0.00 [a]	0.00

Sample sizes refer to the number of individuals measured (at least 3 flowers per individual). Significant differences among species are indicated by different letters ($p<0.001$ following Bonferri Correction, with ANOVA and Tukey's HSD). *Jaltomata* data from [42].

895 **Table 3**. Average length of development, size of 0 day buds (just prior to anthesis), and overall bud growth rates for the five *Jaltomata*
896 species and *S. pimpinellifolium*.

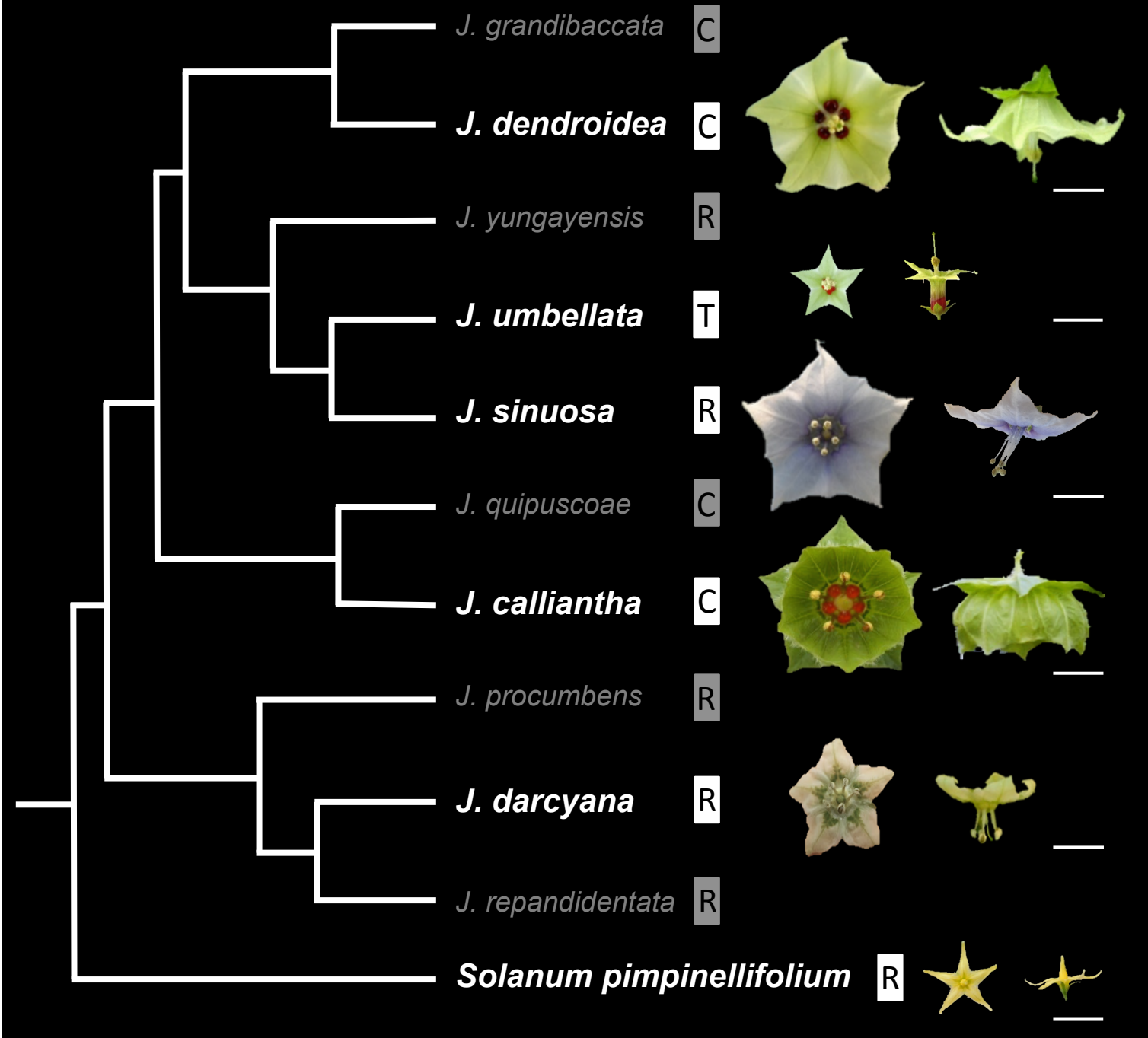
Species	Length (days)		0 Day Bud Diameter (mm)		Growth Rate (mm/day)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
J. darcyana (n=13)	19.23 [a]	1.01	5.00 [a]	0.32	0.261 [a]	0.02
J. sinuosa (n=11)	28.55 [b]	0.93	7.16 [b]	0.45	0.251 [a]	0.02
J. calliantha (n=10)	28.80 [b]	1.03	12.13 [c]	0.36	0.422 [b]	0.02
J. dendroidea (n=5)	32.40 [c]	0.89	15.30 [d]	0.27	0.473 [c]	0.02
J. umbellata (n=13)	18.62 [a]	0.96	3.44 [e]	0.11	0.185 [d]	0.01
S. pimpinellifolium (n=8)	14.00 [d]	0.00	2.75 [f]	0.19	0.196 [d]	0.01

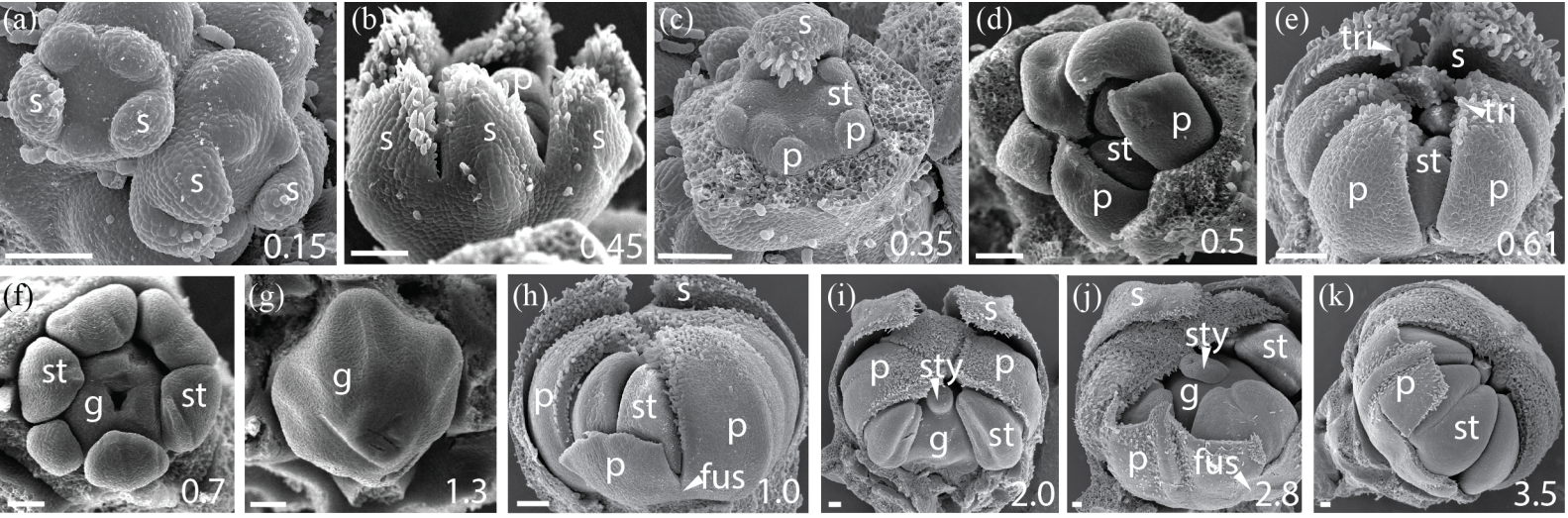
897 Bud diameter was measured every other day, from 1 mm buds to anthesis. Growth rate was determined by dividing bud diameter of day 0 buds by the length in
898 days. Significant differences among species are indicated by different letters (following ANOVA and Tukey's HSD).
899
900
901
902
903
904
905

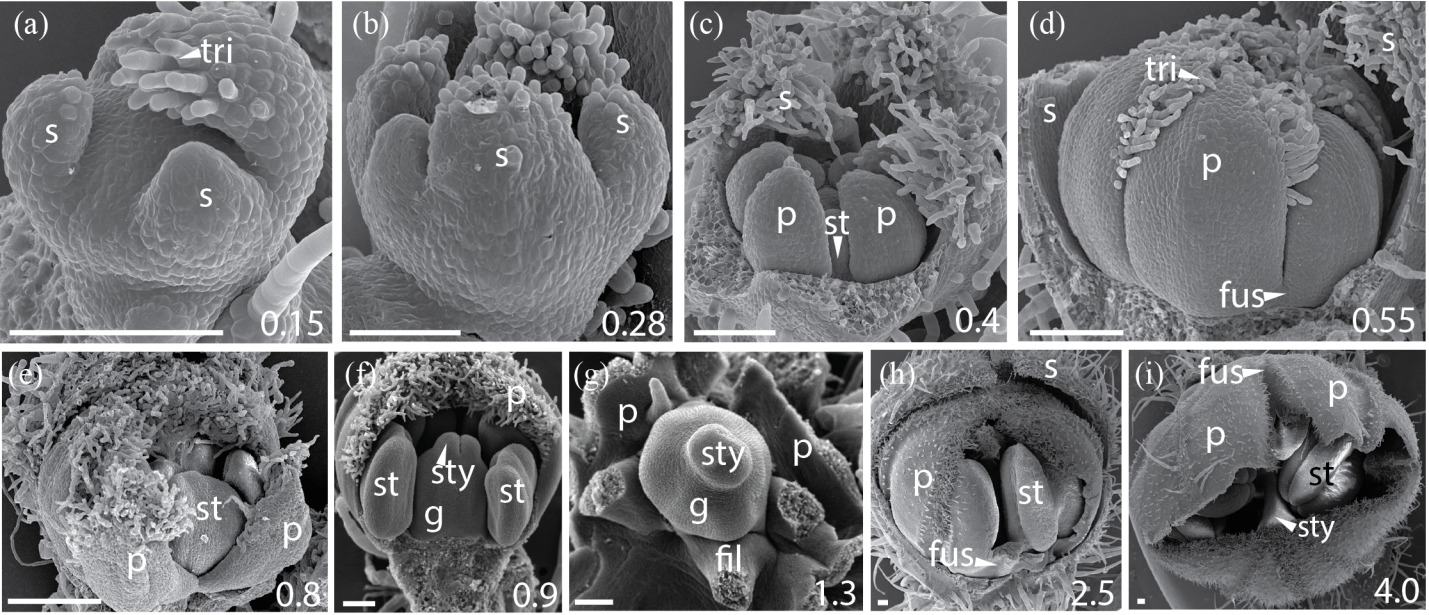
Table 4. Relative growth rates for focal floral organs and features for the examined species, measured against bud diameter, for early and mid + late stages.

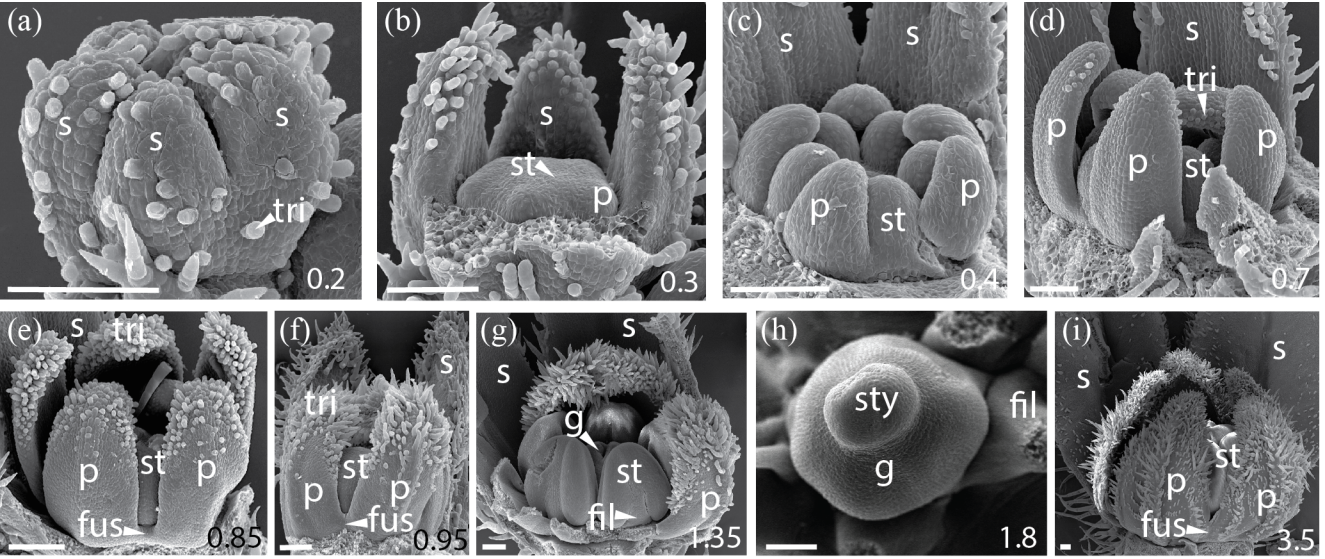
	J. darcyana		***J. sinuosa***		***J. calliantha***		***J. dendroidea***		***J. umbellata***		***S. pimpinellifolium***	
	Early (n=48)	Mid + Late (n=41)	Early (n=35)	Mid + Late (n=41)	Early (n=21)	Mid + Late (n=44)	Early (n=33)	Mid + Late (n=49)	Early (n=35)	Mid + Late (n=58)	Early (n=50)	Mid + Late (n=56)
Sepal Length	0.857	0.788	1.039	0.923	1.281**	1.023*	0.973^	0.880^	1.024	1.329**	2.050***	0.377**
Corolla Depth	0.766	0.982	0.842	1.124	0.964	1.164	0.737^	1.339*^	1.043**	2.742***	1.597***	1.376**
Corolla Fusion	0.110	0.769	0.090	1.195*	0.105	1.330**	0.034*	1.274**	0.164	2.861***	0.437***	0.798
Petal Length	0.845	1.056	0.858	1.323*	0.951	1.262*	0.678^	1.360*	1.019	2.694***	1.696***	1.323*
Lobe Length	0.753	0.690	0.766	0.759	0.866	0.493*	0.601^	0.767^^^	0.892	0.843	1.365***	1.043**
Stamen Length	0.457	0.774	0.505	1.028*	0.521	1.044*	0.459	0.848^^	0.605	2.532***	1.259***	1.306**
Ovary Diameter	0.432	0.445	ND	0.459	0.460	0.728***	0.410	0.477^^^	0.362	0.487	ND	0.298*
Style Length	0.000	0.737	0.000	1.433*	0.000	1.189*	0.000	1.616**^^	0.000	3.440***	0.000	1.899**

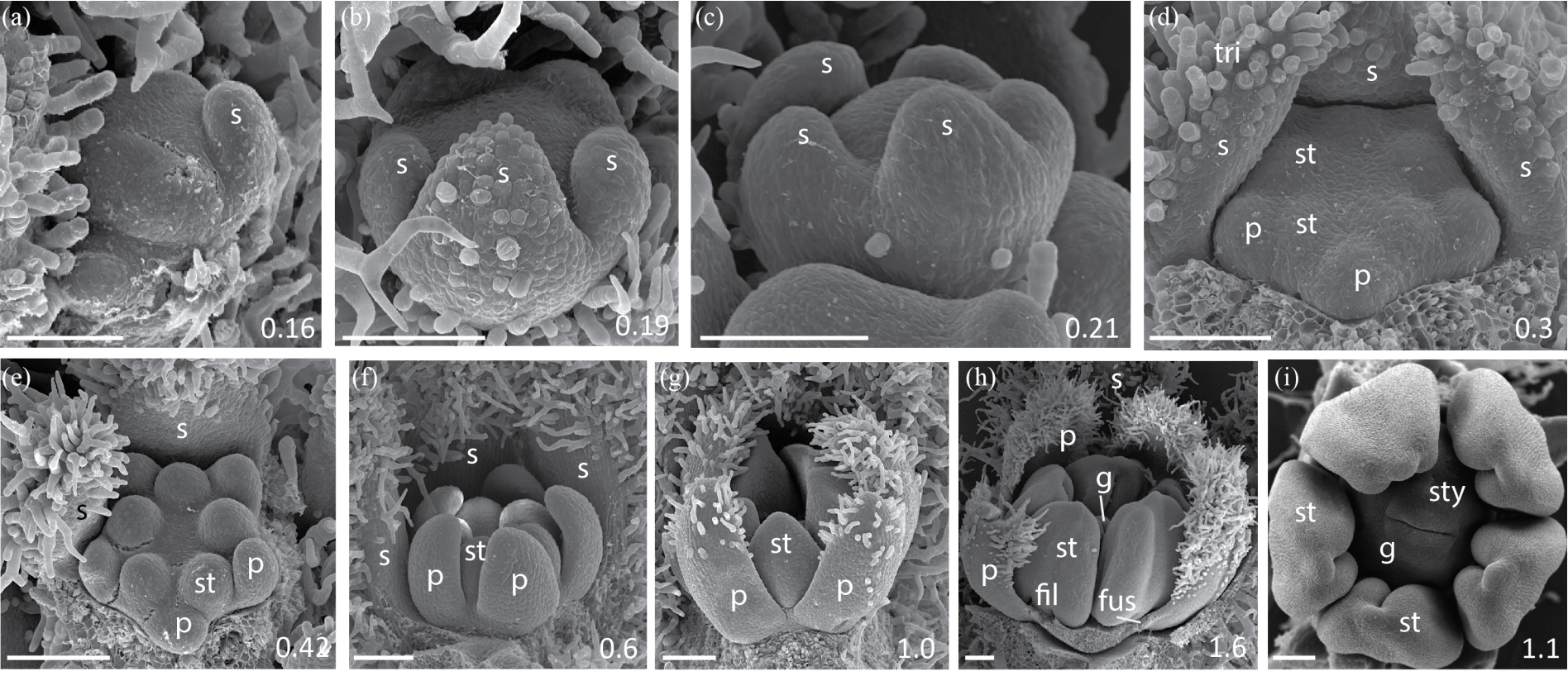
Measurements were log-transformed prior to analysis. Significant differences against *J. darcyana* (ancestral rotate) indicated by * (* $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$); significant differences between *J. calliantha* and *J. dendroidea* (convergent campanulate) indicated by ^ (^ $p < 0.05$; ^^ $p < 0.001$; ^^^ $p < 0.0001$). ND, not determined, as sample sizes were too small.

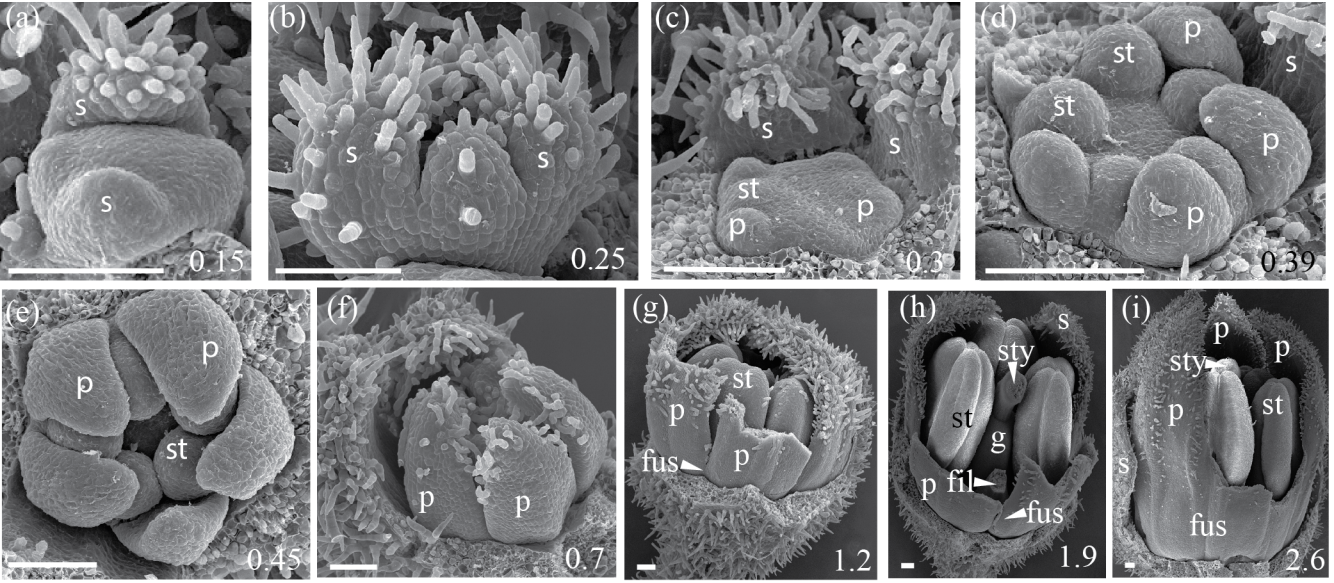

J. grandibaccata
C
J. dendroidea
C
J. yungayensis
R
J. umbellata
T
J. sinuosa
R
J. quipuscoae
C
J. calliantha
C
J. procumbens
R
J. darcyana
R
J. repandidentata
R
Solanum pimpinellifolium
R

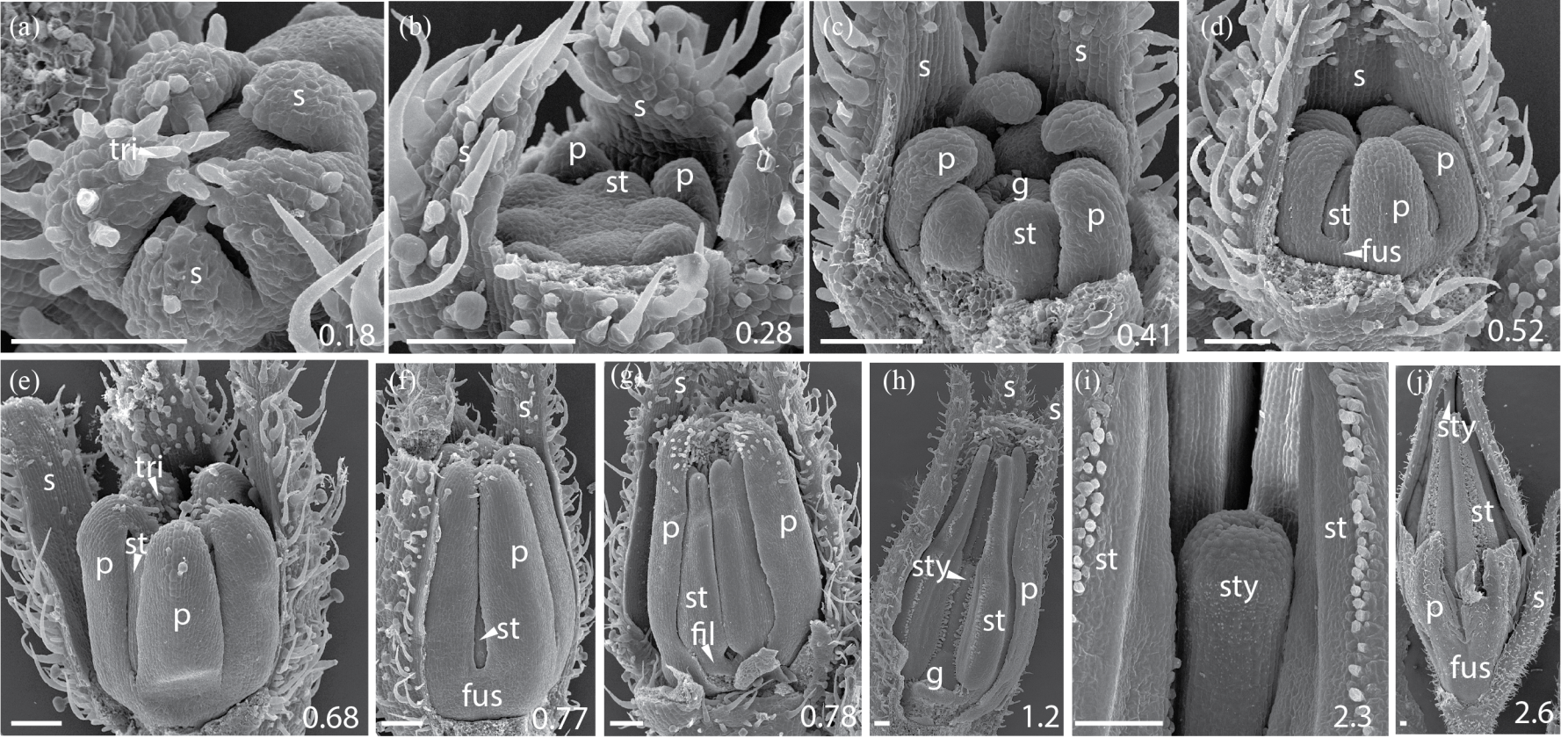

(a)
s
s
s
s
0.15
(b)
s
s
s
0.45
(c)
s
st
p
p
0.35
(d)
p
st
p
0.5
(e)
tri
s
tri
st
p
p
0.61
(f)
st
g
st
0.7
(g)
g
1.3
(h)
s
p
st
p
p
fus
1.0
(i)
s
p
sty
p
g
st
2.0
(j)
s
sty
st
g
p
fus
2.8
(k)
p
st
3.5

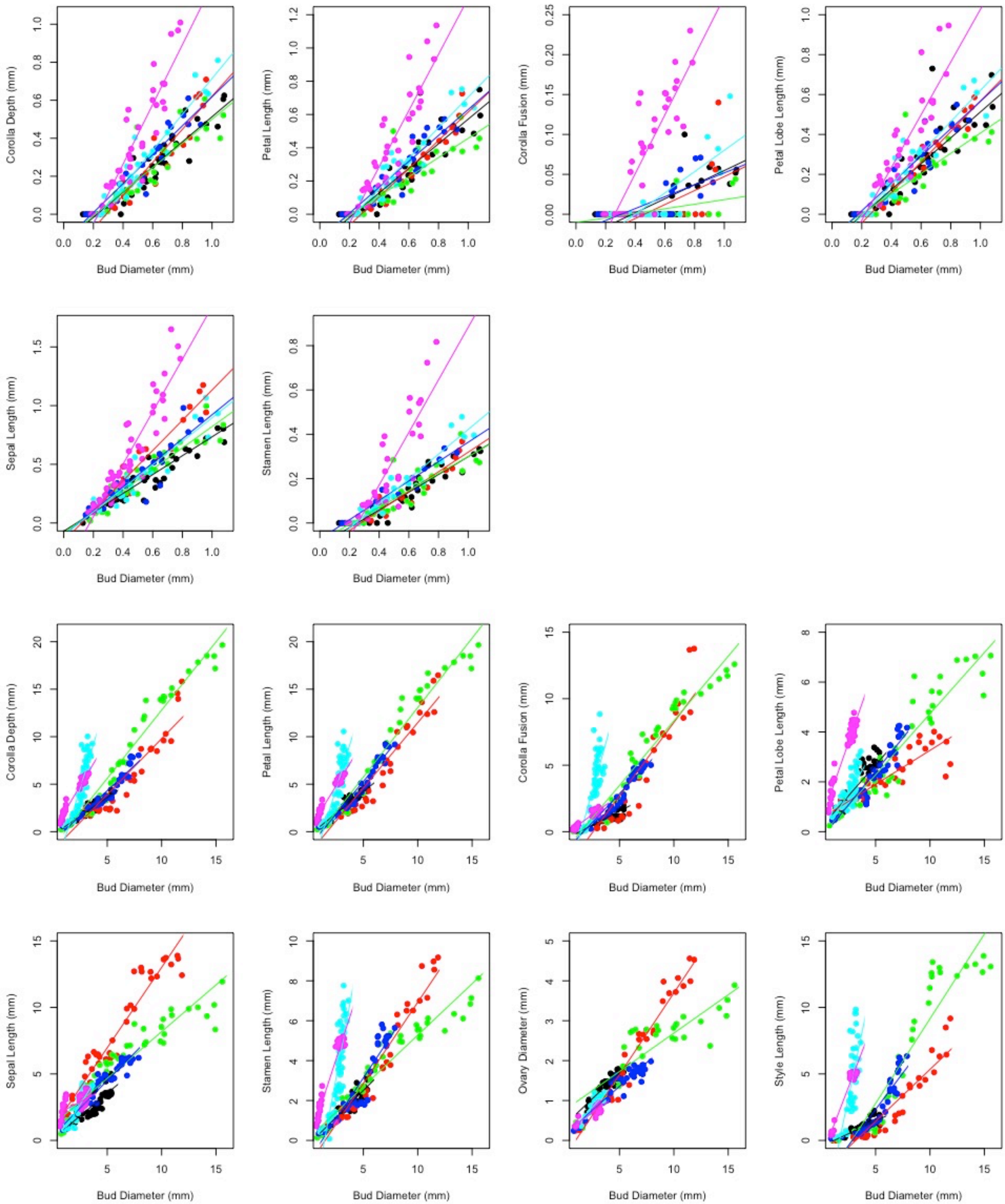

(a)
tri
s
s
0.15
(b)
s
s
0.28
(c)
s
p
st
p
0.4
(d)
s
tri
s
p
fus
0.55
(e)
st
p
p
0.8
(f)
p
st
sty
st
g
0.9
(g)
p
sty
p
g
fil
1.3
(h)
s
p
st
fus
2.5
(i)
fus
p
p
st
sty
4.0


(a)
s
s
s
tri
0.2
(b)
s
st
p
0.3
(c)
s
s
p
st
p
0.4
(d)
s
tri
p
p
st
p
0.7
(e)
s
tri
p
st
p
fus
0.85
(f)
s
tri
st
p
p
fus
0.95
(g)
s
s
g
st
fil
p
1.35
(h)
sty
fil
g
1.8
(i)
s
s
st
p
p
fus
3.5


(a)
s
0.16
(b)
s
s
s
0.19
(c)
s
s
s
0.21
(d)
tri
s
s
st
s
p
st
p
0.3
(e)
s
s
st
p
p
0.42
(f)
s
s
s
st
p
p
0.6
(g)
st
p
p
1.0
(h)
s
p
g
st
p
fil
fus
1.6
(i)
sty
st
g
st
1.1


(a)
s
s
0.15
(b)
s
s
0.25
(c)
s
s
st
p
p
0.3
(d)
p
st
s
st
p
p
0.39
(e)
p
p
st
0.45
(f)
p
p
0.7
(g)
st
p
fus
p
1.2
(h)
s
sty
st
g
p
fil
fus
1.9
(i)
p
sty
p
p
st
s
fus
2.6


(a)
s
tri
s
0.18
(b)
s
s
p
st
p
0.28
(c)
s
s
p
g
st
p
0.41
(d)
s
p
st
p
fus
0.52
(e)
s
tri
st
p
p
0.68
(f)
s
p
st
fus
0.77
(g)
s
p
p
st
fil
0.78
(h)
s
s
sty
p
st
g
1.2
(i)
st
st
sty
2.3
(j)
sty
st
p
s
fus
2.6


Corolla Depth (mm)
Petal Length (mm)
Corolla Fusion (mm)
Petal Lobe Length (mm)
Sepal Length (mm)
Stamen Length (mm)
Corolla Depth (mm)
Petal Length (mm)
Corolla Fusion (mm)
Petal Lobe Length (mm)
Sepal Length (mm)
Stamen Length (mm)
Ovary Diameter (mm)
Style Length (mm)
Bud Diameter (mm)