Genetic control of floral size and proportions

JULIA WEISS, LUCIANA DELGADO-BENARROCH and MARCOS EGEA-CORTINES*
Área de Genética, Departamento de Ciencia y Tecnología Agraria, Escuela Técnica Superior de Ingeniería Agronómica, Universidad Politécnica de Cartagena, Cartagena, Spain

ABSTRACT  Floral size is an ecologically important trait related to pollination success and genetic fitness. Independently of the sexual reproduction strategy, in many plants, floral size seems to be controlled by several genetic programs that are to some extent independent of vegetative growth. Flower size seems to be governed by at least two independent mechanisms, one controlling floral architecture that affects organ number and a second one controlling floral organ size. Different organ-dependent growth control may account for the final proportions of a flower as a whole. Genes controlling floral organ identity, floral symmetry and organ polarity as well as auxin and gibberellin response, also play a role in establishing the final size and architecture of the flower. The final size of an organ seems to be controlled by a systemic signal that might in some cases overcome transgenic modifications of cell division and expansion. Nevertheless, modification of basic processes like cell wall deposition might produce important changes in the floral organs. The coordination of the direction of cell division and expansion by unknown mechanisms poses a challenge for future research.

KEY WORDS: floral meristem, cell cycle, systemic signal, floral patterning, floral architecture

The final shape and body size of multicellular organisms is the result of a genetic program and the influence of environmental conditions. In animals and plants the intrinsic growth rate is modulated by nutrient availability that determines the final size of the organism. In animals, both body size and longevity are to some extent controlled by the insulin pathway that is in itself dependent on nutrient conditions (Nijhout, 2003). But one important difference between plants and animals is that in plants, the formation of the different organs happens after embryonic development, thus not only organ or body size is influenced by environmental clues but also the types of organs produced. Our understanding of the way final plant size is achieved has been obtained using two different approaches: physiologists have tried to understand the roles of the so called plant growth regulators and environmental signals on plant development whereas geneticists have concentrated their efforts in finding mutants, genes or natural variation affecting growth in any of its forms. Although these two research lines appear separate, the reality is that they have been linked by an enormous amount of work done by plant breeders studying gene and environment interactions on agricultural traits that are related to growth, like yield, fruit size, biomass production etc. The efforts done in the model system Arabidopsis have helped to bring together the more basic approaches since mutations affected in plant growth regulator synthesis, degradation and the transduction of the signal have been isolated and characterized.

Which are the mechanisms that control the final size of an organism is a question without a clear answer yet. There are two basic processes that could contribute to its control: cell division and expansion but the integration of these two cellular programs into the context of organ growth and development is poorly understood (Torii, et al., 1996). Plant growth and determination of the final size of plant organs is a modular process, happening throughout the entire lifespan in response to intrinsic developmental patterns and external conditions (Doonan, 2000). Both cell division and cell expansion contribute to organ growth and factors determining the integration of these two cellular processes into the context of organ growth and development are the topic of many investigations both in animals and plants (Day and Lawrence, 2000, Potter and Xu, 2001).

Despite the enormous amount of knowledge accumulated in Arabidopsis by cloning and genetic analysis of developmental mutants in most cases phenotypic descriptions of flowers tend to be scarce, suggesting that either some of the mutants have subtle floral phenotypes, or that the effect is too complex to be described in simple terms. In this review we discuss the recent advances in the understanding of the control of organ size and proportions with a special emphasis on floral size in different model systems.

*Address correspondence to: Dr. Marcos Egea-Cortines. Área de Genética, Departamento de Ciencia y Tecnología Agraria, Escuela Técnica Superior de Ingeniería Agronómica, Universidad Politécnica de Cartagena, 30203 Cartagena, Spain. e-mail: marcos.egea@upct.es
Ecological and evolutionary significance of floral size and proportions

Most plants can be roughly classified into two different reproductive strategies, those that largely rely on outcrossing with other members of the species (allogamy) and those that tend to fertilize themselves (autogamy). In allogamous species, the dependence in some cases on pollinators for reproduction has led to the development of ecological traits that have a function in attraction of birds, insects, bats etc. A detailed review about the evolution of color and related traits has been published recently by Clegg and Durbin (Clegg and Durbin, 2003). One of the traits that play a role in pollination of allogamous species is floral size. Floral size is studied under the so called mating system, since in many cases there is some kind of coevolution between floral size traits and pollinators. For instance, larger corollas seem to attract more pollinators and in this respect there might be an advantage for larger petals, but studies comparing hermaphroditic plants with gynodioecious plants (having either female or hermaphroditic plants) show that hermaphroditic plants tend to have smaller flowers probably due to preferential allocation of energy into fruit and seed formation (see Miller and Venables and references therein) (Miller and Venable, 2003). The general picture suggests that there is a strong genetic control over floral size and the relative growth of the organs, timing to anthesis and coincidence in time and space of fertile pollen and receptive stigma.

Most of the advances in our understanding of floral size and its control at the molecular level have come from Arabidopsis and Antirrhinum; two species with quite opposite reproduction strategies. Arabidopsis is a selfing annual (Somerville and Koornneef, 2002) that is partially cleistogamous whereas Antirrhinum in nature is a perennial and strictly allogamous (Lai et al., 2002, Qiao et al., 2004). The Antirrhinum research lines however are self pollinators (Xue et al., 1996) due to mutation of the S-locus. This difference might be apparent in the future since it is currently difficult to predict the degree of conservation between genes controlling floral organ size in a plant like Antirrhinum, where floral size traits might be under strong evolutionary pressure and Arabidopsis where floral size might not play a major role in fertilization.

Different degrees of coupling between floral and vegetative traits and among floral characters was already observed by Berg (Berg, 1959). In plants with specialised pollination systems, flower parameters were observed to vary less than vegetative traits with a tighter phenotypic integration of floral characters than in plants with unspecific mating systems. Later investigations found that a de-coupling of vegetative from floral traits is species specific and can also be found in plants with unspecialised pollination systems (Armbuster et al., 1999). A tight coupling between floral and vegetative traits could be explained either by an environmental correlation, that means a common response to an environmental cue, or by genetic correlation, that means a common inheritance due to a pleiotropic effect of a single gene on a set of developmentally related traits (see below) or a linkage disequilibrium between separate genes with effect on different characters (Juenger et al., 2000). An additional way to separate vegetative and floral gene functions is by allele specific interactions, recently shown in a cincinnati allele in Antirrhinum (Crawford et al., 2004) (see below).

In Antirrhinum, we have been studying different mutations that affect floral size and proportions and we have found that the classic mutations compacta, muscoides (Stubbe, 1966) and nanalargiflora (Stubbe, 1974) and the newly identified ktana (Weiss and Egea-Cortines, unpublished), affect both vegetative and floral development whereas the classic mutants compacta ähnlich, formosa, Grandiflora and Nitida seem to affect only the flower under normal growth conditions. This suggests that at least two sets of genes that control floral size and proportions, one that has functions both in vegetative and reproductive growth and a second one that is probably flower specific. Natural variation is a great resource in Antirrhinum since there are more than 16 wild species that can be crossed with each other. In an F2 of A. majus 165E line (Sommer et al., 1985) with the wild A. majus ssp linkianum we have found segregation of floral size in plants that show nearly identical leaf size suggesting that there is a degree of separation between genes controlling leaf expansion and floral size (Fig. 1). The analysis of natural variation in a recombinant inbred line built from A. majus and the wild species A. charidemi shows that several QTL controlling floral size are specific for the flower, confirming a partial separation of vegetative and reproductive control (A. Hudson personal communication). A recent survey of QTL controlling leaf, sepal and petal size in tomato has shown that there is no overlap between QTL controlling the same trait in different organs (Frary et al., 2004). These results seem to be also true in Arabidopsis where a comparison of QTL affecting leaf and floral development have found eleven QTL that affect only one of the organs and two that have pleiotropic effects (Juenger et al., 2005). The partial isolation of the flower from the rest of the plant in terms of regulatory genes might be true even for some basic processes like sugar sensing, that is central to plant development (Leon and Sheen, 2003). For instance the loss of function of the glucose sensor hexokinase glucose insensitive 2 (gin2) in Arabidopsis causes extreme dwarfism but floral size is apparently normal (Moore et al., 2003).

From a developmental perspective, it is generally agreed that the activation of the major switch that causes the shoot apical

Fig. 1. Separation of vegetative and floral growth traits. Pictures corresponding to F1 plants of a cross between A. majus and A. linkianum showing the third leaf and first flower of three different plants segregating floral size, but showing similar leaf size.
meristem (SAM) to produce floral primordia instead of leaves or branches is the FLORICAULA (FLO) gene in Antirrhinum (Coen et al., 1990), LEAFY (LFY) in Arabidopsis (Weigel et al., 1992), FALSIFLORA in tomato (Moliner-Rosales et al., 1999) or ABERRANT LEAF NON FLOWER in Petunia (Souer et al., 1998). Ectopic expression of LFY or its orthologs in the shoot apical meristem causes increased flowering speed in many systems like Arabidopsis, poplar (Weigel and Nilsson, 1995) or orange trees (Pena et al., 2001), but does not cause ectopic floral tissues to develop outside floral primordia. This suggests that the floral context, understood as the specific transcriptome and proteome that leads to the formation of inflorescence primordia, is largely, but not completely, controlled by the FLO/LFY switch. Indeed in some plants like tobacco or vine, the FLO/LFY ortholog is expressed in vegetative meristems suggesting an evolutionary divergence (Carmona et al., 2002, Kelly et al., 1995). Thus it is formally possible that the observed genetic separation between floral and vegetative traits might be a genetic program activated by the floral context, or is an intrinsic part of it in those species that have this characteristic.

Genes controlling floral size and architecture

The control of floral size can be separated into two different aspects, one is the control of the number of organs in a whorl, thus affecting floral size in terms of sheer number of organs and a different mechanism is that controlling the size of each of the organs formed within a flower. The flower, like the rest of the organs of higher organisms, has a certain "normal size" in a species. During plant development, after transition from vegetative to reproductive growth, the SAM produces flowers instead of leaves and there are different sets of genes that regulate cellular mechanisms during each developmental step. As floral development proceeds, local regions of cell division establish individual floral organ primordia at specific distances and angles from each other. This process is controlled by three classes of genes, those that affect development of floral primordia, those that alter floral symmetry and those that specify organ identity (Schwarz-Sommer et al., 1990). Cell number in the centre of the meristem of Arabidopsis is, among others, regulated by the genes CLV and WUS (Clark et al., 1997, Haecker et al., 2001, Irish and Jenik, 2001, Kayes and Clark, 1998, Laufs et al., 1998, Mayer et al., 1998). The cell number in floral primordia, the spacing of organ inception, the determination of organ shape and the specification of organ size are all controlled separately (Meyerowitz, 1997). A proposed model of determination of floral size based on the data described below and our own observations is shown in Fig. 2. A list with some of the genes controlling floral size and proportions can be seen in Table 1.

Mutants that affect the number of floral organs include PERIANTHIA (PAN) (Chuang et al., 1999, Running and Meyerowitz, 1996), ETTIN (ETT) (Sessions et al., 1997), WIGGUM (WIG) (Running et al., 1998) or the SUPERMAN gene of Arabidopsis (Huang and Ma, 1997, Jacobsen and Meyerowitz, 1997). The mutants pan and ett have in common an increase in sepals and petals and decrease in stamen numbers whereas sup produces more stamens at the expense of carpels. In contrast wig has more organs in all the whorls and shows synergistic interactions with other genes controlling cell division in primordia like CLV.

Gene mutations which control the overproliferation of cells in the SAM like the three CLAVATA genes show similar phenotypes with increased organ number in the flower and modified floral architecture (Brand et al., 2000, Clark et al., 1993, Clark et al., 1997, Fletcher et al., 1999). The size of the floral anlage seems to play a role in the number of organs that are formed since several genes are involved in restricting the accumulation of cells in floral primordia. For instance the ULTRAPETALA (ULT) mutation has more organs than wild type (Fletcher, 2001) and it seems to control the size of the floral meristem through control of CLV expression and repression of WUS activity (Carles et al., 2004).

A large number of mutants affect floral architecture via effects on general cell division, meristem size, or cell allocation to primordia. Plants affected in the STRUWELPETER gene (SWP) show smaller organs in the aerial part of the plant and reduced organ number in the flower, leading to abnormal architecture (Autran et al., 2002). Genes involved in stem cell formation like...
architecture is compromised. Required to form an organ and below this critical number, floral primordia suggesting that there is a minimal threshold of cells required to form an organ and below this critical number, floral architecture is compromised.

Floral homeotic genes

The observation of many of the classical floral homeotic mutants in Antirrhinum and Arabidopsis has led to the conclusion that all the major genes involved in the establishment of floral organ identity exert some control over growth in the flower in one way or another. Thus the floral organ identity genes DEFINITION-APE1TALA3 (Jack et al., 1992; Sommer et al., 1990), GLOBOSA-PISTILLATA (Goto and Meyerowitz, 1994; Trobner et al., 1992) and PLENA-AGAMOUS (Bradley et al., 1993; Yanofsky et al., 1990) from Antirrhinum and Arabidopsis show effects on floral organ size.

Weak alleles of the B function gene deficiens like defnicotianoides or defchlorantha show decreased petal development and defnic stamens are shorter than in wild type (Sommer et al., 1990) (see Fig. 3A). The temperature sensitive allele def101 shows petals close to wild type organ size at 15ºC as compared to 25ºC when petal organs are absent and transformed into sepals (Zachgo et al., 1995). Thus, although the primary function of B

<table>
<thead>
<tr>
<th>Effect</th>
<th>Gene</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased organ number</td>
<td>CLAVATA 1, 2 and 3</td>
<td>Arabidopsis</td>
<td>(Clark et al., 1995; Fletcher et al., 1999; Kayes and Clark, 1998)</td>
</tr>
<tr>
<td></td>
<td>ULTRAPETALA</td>
<td>Arabidopsis</td>
<td>(Fletcher, 2001)</td>
</tr>
<tr>
<td></td>
<td>WIGGUM</td>
<td>Arabidopsis</td>
<td>(Running et al., 1998)</td>
</tr>
<tr>
<td></td>
<td>CYCLOIDEA</td>
<td>Antirrhinum</td>
<td>(Luo et al., 1996)</td>
</tr>
<tr>
<td></td>
<td>JAGGED</td>
<td>Arabidopsis</td>
<td>(Drinnen et al., 2004; Ohno et al., 2004)</td>
</tr>
<tr>
<td>Increased organ number - control of C function</td>
<td>AGAMOUS-PLENA</td>
<td>Arabidopsis, Antirrhinum</td>
<td>(Bowman et al., 1989; Bradley et al., 1993)</td>
</tr>
<tr>
<td></td>
<td>POLYPETALA</td>
<td>Antirrhinum</td>
<td>(McSteen et al., 1998)</td>
</tr>
<tr>
<td>Change in relative organ numbers</td>
<td>FLORIPETALA</td>
<td>Arabidopsis</td>
<td>(Running et al., 2004)</td>
</tr>
<tr>
<td></td>
<td>SUPERMAN</td>
<td>Arabidopsis</td>
<td>(Huang and Ma, 1997; Jacobsen and Meyerowitz, 1997)</td>
</tr>
<tr>
<td></td>
<td>PERIANTHIA</td>
<td>Arabidopsis</td>
<td>(Chuang et al., 1999; Running and Meyerowitz, 1996)</td>
</tr>
<tr>
<td></td>
<td>ETTIN</td>
<td>Arabidopsis</td>
<td>(Sessions et al., 1997)</td>
</tr>
<tr>
<td>Decrease in floral organ number</td>
<td>WUSCHEL</td>
<td>Arabidopsis</td>
<td>(Lau et al., 1996; Mayer et al., 1998)</td>
</tr>
<tr>
<td></td>
<td>STRUWELOPETAL</td>
<td>Arabidopsis</td>
<td>(Auran et al., 2002)</td>
</tr>
<tr>
<td></td>
<td>DEFICIENS</td>
<td>Antirrhinum</td>
<td>(Sommer et al., 1990)</td>
</tr>
<tr>
<td></td>
<td>LEUNIG</td>
<td>Arabidopsis</td>
<td>(Liu and Meyerowitz, 1995)</td>
</tr>
<tr>
<td></td>
<td>STEREAL APETAL</td>
<td>Arabidopsis</td>
<td>(Byzova et al., 1999)</td>
</tr>
<tr>
<td></td>
<td>APETALA2</td>
<td>Arabidopsis</td>
<td>(Crone and Lord, 1994; Kunst et al., 1989; Maes et al., 1999)</td>
</tr>
<tr>
<td></td>
<td>PIN FORMED-1</td>
<td>Arabidopsis</td>
<td>(Bennett et al., 1995, Okada et al., 1991)</td>
</tr>
<tr>
<td></td>
<td>PINOID</td>
<td>Arabidopsis</td>
<td>(Bennett et al., 1995)</td>
</tr>
<tr>
<td></td>
<td>FLOOZY</td>
<td>Petunia</td>
<td>(Tobera-Santamaria et al., 2002)</td>
</tr>
<tr>
<td></td>
<td>ANTIGUMENTA</td>
<td>Arabidopsis</td>
<td>(Elliott et al., 1996; Klucher et al., 1996)</td>
</tr>
<tr>
<td></td>
<td>FILAMENTOUS FLOWER</td>
<td>Arabidopsis</td>
<td>(Chen et al., 1999; Sawal et al., 1999)</td>
</tr>
<tr>
<td></td>
<td>SKP</td>
<td>Arabidopsis</td>
<td>(Ni et al., 2004)</td>
</tr>
<tr>
<td></td>
<td>UNUSUAL FLORAL ORGANS</td>
<td>Arabidopsis</td>
<td></td>
</tr>
</tbody>
</table>
function genes is the control of organ identity, they also play a role in activating petal and stamen growth. Loss of B function genes in Arabidopsis causes loss of organs in the third whorl for instance in pi-1 (Bowman et al., 1991) and the ectopic expression of AP3 and P' leads to the rescue or organ number in class A mutants and an increased number of stamens due to increased whorl number (Krizek and Meyerowitz, 1996) suggesting that B function genes play a dual role in controlling cell proliferation both in the formation of primordia and in organ growth in Arabidopsis. The activation of both B and C function genes is controlled by the F-box protein FIMBRIATA (Fim) (Ingram et al., 1997; Simon et al., 1994) and its Arabidopsis ortholog UNUSUAL FLORAL ORGANS (Ufo) (Ingram et al., 1995; Levin and Meyerowitz, 1995; Wilkinson and Haughn, 1995). Ectopic expression of UFO in Arabidopsis causes increased floral organ size (Lee et al., 1997) due to increased cell division (Mizukami, 2001). The fact that ectopic expression of B function genes does not cause an increase in floral organ size suggests that the increase resulting from UFO misexpression is not due to the higher B function activity observed, but to other unknown factors. This would be in agreement with the results of Ingram et al., that suggest that FIM acts by selective degradation of regulatory proteins involved in controlling floral homeotic gene functions and cell division (Ingram et al., 1997). Proteins of the F-box family can bind proteins of the SKP family and form complexes with target proteins that are degraded by the ubiquitin pathway (Ciechanover, A. 1998). Recent experiments show that loss of function of the UFO partners also cause major disruption of floral development including loss of organs and arrested petal development (Ni et al., 2004).

The C function genes are involved in controlling organ identity and meristem determinacy in Antirrhinum (Bradley et al., 1993), Arabidopsis, (Yanofsky et al., 1990) tomato, (Pnueli et al., 1994), petunia and cucumber (Kater et al., 1998). Loss of C function activity results in formation of additional whorls in the inner part of the flower, substituting the carpels with a reiteration of sepal, petal, petal whorls in Antirrhinum and Arabidopsis (Davies et al., 1999). Reduced activity of genes that are required to activate or maintain C function like POLYPETALA, also show increased organ number due to additional whorls of petals (McSteen et al., 1998). This increase in organ number is caused by the maintenance of WUS in the floral meristems that in normal conditions would be repressed by the C function (Lenhard et al., 2001; Lohmann et al., 2001). The genetic analysis of CLV, ULI, WUS and AG show that WUS plays an indirect but important role in establishing the number of cells available to form floral primordia at early stages, before its inactivation by C function to terminate the flower.

The classical Arabidopsis A function genes controlling floral organ identity like AP2 (Crone and Lord, 1994; Kunst et al., 1989; Maes et al., 1999) or other repressors of C function expression in the perianth like LEUNIG (Liu and Meyerowitz, 1995) or STERILE APETALA (Byzova et al., 1999) have strong effects in the architecture of the flower. Ectopic expression of the Arabidopsis AP2 in Petunia causes flowers with increased organ number (Maes et al., 1999). Since the A function is a negative regulator of the expression of AGAMOUS and AG is known to inhibit WUS (Lohmann et al., 2001), it is formally possible that the observed loss of organs in A function mutants is caused by the premature repression of WUS by ectopic AG expression. Similarly the increase in organ number by ectopic AP2 expression could result from inhibition of AG and therefore increased WUS activity.

In spite of considerable knowledge of the function of these genes in Arabidopsis, the situation seems to be different in Antirrhinum and Petunia. Petunia has three AP2-like genes, one of them can complement the Arabidopsis mutation and the existence of three paralogs has limited tests of functional conservation (Maes et al., 2001). Furthermore the two Antirrhinum AP2 orthologs, known as LIPLLESS do not show loss of organ number in double mutant combinations (Keck et al., 2003) (see below).

Auxins and floral development

Auxins play an important role in lateral organ formation and early floral development (Bennett et al., 1995; Okada et al., 1991). Detailed analysis of mutations that resemble Arabidopsis plants grown in the presence of auxin transport inhibitors suggest that the so-called pin group of mutations, generally affected in lateral organ primordia initiation and floral patterning, are in fact mutants affected in auxin transport and signalling. Mutations in PIN1 or PID develop flowers that display a decrease in the number of sepals and stamens and an increase in petals suggesting that PID plays a role in the formation of organ primordia (Bennett et al., 1995). The overexpression of PID causes a strong defect in patterning including lack of lateral organs that is thought to be the result of a shift of apical-basal targeting of the PIN1 protein (Frimer et al., 2004). Recent work has identified auxin synthesis genes like the Yucca gene from Arabidopsis that encodes a flavin monooxygenase (Zhao et al., 2001). Its Petunia ortholog, FLOOZY (FZY), plays a major role in floral architecture since fzy mutants lack in most cases the organs of the outer three whorls (Tobeña-
Santamaria et al., 2002). However it is interesting that in fzy mutants SEM analysis of the floral meristems show that organ patterning is correct and the lack of organs is due to a failure of the different primordia to grow. Although the current data support the hypothesis that auxins play a major role in primordia initiation (Reinhardt et al., 2003, Reinhardt et al., 2000), the results suggest that in flower development auxins might be dispensable for the initiation of floral primordia, or in fzy mutants there is a mechanism that allows enough auxin to accumulate to produce primordial but not to maintain organ growth.

The data from the above genes show that the total number of cells allocated to form floral primordia is important to maintain floral architecture. Since each organ seems to achieve more or less wild type size in many mutants affected in floral architecture, the number of cells allocated to form floral organ primordia might be maintained in the mutants and a deficit or excess of cells translated into a modified number of organs. All together, these data suggest that cell number is a factor controlling organ size since an unknown mechanism seems to allocate a minimum amount of cells to each primordium that ensures proper organ formation. In those cases where cell division after primordia initiation is also compromised, organ size might also be affected (see below).

Genes that affect floral symmetry

One of the main differences in floral architecture in angiosperms is the existence of two main kinds of flowers, those with a zygomorphic symmetry like Antirrhinum and those with radial symmetry like Arabidopsis, Petunia or tomato (Coen and Nugent, 1994). Pioneering work in Antirrhinum has shown that the genes involved in establishment of the zygomorphic symmetry like CYCLOIDEA (CYC) (Luo et al., 1996) or DICHOTOMA (Luo et al., 1999) belong to the TCP family of transcription factors (Cubas et al., 1999). Mutants in the CYC locus have five stamens since the adaxial stamen that in wild type aborts grows to normal size. In situ hybridization experiments show that CYC inhibits the cell cycle genes CYCLIN D3b and HISTONE H4 in the position of the staminoids whereas in cyc mutant plants, expression of the cell cycle genes predicts formation of a functional organ (Gaudin et al., 2000). Altogether these data confirm the general idea that organs cannot form without cell division.

Genes that affect floral organ size

A way to analyse the regulation of organ size is by studying the pattern of cell division and expansion during development and how they are affected in mutants that show modifications in leaves and/or flowers (Tsuge et al., 1996). Studies of mutations with pleiotropic effects frequently describe morphological or cellular changes in the leaves, whereas the effect on other shoot organs, especially the different floral organs, is treated less profoundly (Hu et al., 2003; Smith et al., 1996; Tsuge et al., 1996; Tsukaya, 2003; Wyrzykowska et al., 2002; Wyrzykowska and Fleming, 2003). We review below some of the genes for which detailed descriptions of floral phenotypes are available.

The AINTEGUMENTA mutation was isolated independently by two groups as a female sterile mutation in Arabidopsis (Elliott et al., 1996; Klucher et al., 1996). The ANT locus encodes a transcription factor of the AP2 family and the loss of function of the gene causes severe disruption of ovule development. The defects include lack of integument, a block of embryo sac formation and arrest of megagametogenesis. The development of the floral organs is also strongly affected, showing random loss of organs in the outer three whorls and a strong effect on organ growth. Amongst the phenotypes described, the ant-9 allele shows serrated sepals, narrow petals, thin anthers, sepaloid and petalloid stamens and unfused gynoecium (Elliott et al., 1996), whereas ant-1 shows similar phenotypes but petals width is more often affected than length (Klucher et al., 1996). AINTEGUMENTA is also expressed in vegetative primordia (Elliott et al., 1996) and roots (Klucher et al., 1996) and plants homozygote for ant-1 have smaller leaves than wild-type. The random losses of organs in ant mutants suggest that the general cell division process is severely disrupted and, as we proposed above, both floral anlagen and further organ development seem to be affected. The ant gene has an additional function promoting petal identity that might also explain the decrease in petal size in ant mutants (Krizek et al., 2000).

The overexpression of ANT in Arabidopsis causes increase in organ size in the flower. The increase in organ size is due either to increased cell division in sepals and cell expansion in the inner three whorls (Krizek, 1999), or to increased cell division in petals too (Mizukami and Fischer, 2000). Mizukami and Fischer also found increased vegetative growth. Plants overexpressing ANT produce larger leaves as the result of an extended period of leaf growth caused by a longer period of cell division. Consistent with this observation, cyclin D3 and histone H4 were found to be expressed ectopically in sepals of 35S::ANT flowers. AINTEGUMENTA is thought to promote growth at different levels, both in the shoot apical meristem as well as within floral primordial and in floral organs. The results discussed above suggest that both rate and duration of cell division are important to determine the final size of both lateral and floral organs.

Auxins play a role in establishment of primordia and expansion of lateral organs. The gene ARGOS was identified in a microarray experiment studying the effect of the auxin naphthylacetic acid (NAA) on roots of 7-day old Arabidopsis plants (Hu et al., 2003). Its expression is not confined to roots and it can be detected in aerial parts of the plant, stems, rosette and flowers. The cellular localization of the GFP fusion shows a general distribution of the protein throughout the cell, but the molecular function of the protein remains to be established. Manipulation of ARGOS gene expression by antisense and overexpression shows that it plays a role in general organ growth since antisense plants have smaller organs in the aerial parts, including leaves, flowers and fruits whereas plants with increased ARGOS mRNA have larger leaves, flowers and siliques. Moreover, the overall plant size corresponds to the single organ phenotypes suggesting that ARGOS may act in the general signal transduction pathway of auxins involved in growth control. A detailed analysis of 35S::ARGOS plants shows that the increased size of the organs is due to an extended period of cell division and not an increase in growth rate. One possible explanation for this phenomenon is the observed overexpression of ANT in 35S::ARGOS, that suggests that ARGOS acts via activation of the ANT gene. This hypothesis seems to be correct since the increased growth observed in 35S::ARGOS is abolished in an ant-1 genetic
background. ARGOS acts downstream of some auxin genes like AUXIN RESISTANT AXR1 since 35S:ARGOS can partially restore organ development in axr1 mutants, suggesting that auxins may act in promoting general growth via ARGOS and ANT defining the timing of cell division in all aerial primordia.

The classical mutation erecta has a compact inflorescence, round leaves with short petioles and short and round siliques (Tori et al., 1996). The erecta mutation has no effect on floral organs, but this is due to genetic redundancy with ERECTA-like genes. The ERECTA gene codes for a receptor-like kinase and there are at least seven genes similar to ERECTA and over 600 receptor kinases in the Arabidopsis genome. Loss of function alleles in the two ERECTA-LIKE genes (ERL1 and ERL2) do not show an apparent phenotype, but double mutants with er enhance some of the aspects of the mutation like shorter siliques but stamen and carpel numbers are similar to wild type (Ohno et al., 2004). Petals in plants homozygous for jag are shorter than the wild type and are serrated in the distal part (thus the name of the gene) and a weak serration can be seen in leaves. Studies of cell size (Ohno et al., 2004) and distribution of dividing cells using in situ hybridization (Dinneny et al., 2004a), show that both cell number and shape are affected in the mutant. The JAG gene seems to be required to promote growth of lateral organs and the coincidental expression of JAG with GUS driven by the Cyclin1At promoter suggests that JAG might be necessary to maintain cell division in the margins of organs. The ANT and ARG genes are also involved in maintenance but in contrast to ANT or ARG, JAG overexpression does not cause increased organ size and the main phenotype seen is the development of leaf organs instead of flowers, suggesting that JAG and ANT do not share target genes.

An additional clue to the compartment hypothesis of floral organ development is given by the LIPLESS genes that were identified in Antirrhinum using a reverse genetics approach to study the orthologs of the Arabidopsis A function gene APETALA2 (Coen and Meyerowitz, 1991) (Jofuku et al., 1994) (Keck et al., 2003). APETALA2 is a member of a large family of transcription factor genes (Okamura et al., 1997). Antirrhinum has two genes with close homology to AP2 and only lip1, lip2 double mutants show a novel phenotype in which the lips of the Antirrhinum flower fail to develop. The petal cells showed a morphology that is somewhat different from the regular flat cells present in the proximal part of the lips or the conical cells of the distal part (Glover et al., 1998, Noda et al., 1994). Petal growth is strongly reduced and reduction of the inner organs include shorter stamens and style, but the ovary is twice the length of the wild type (Keck et al., 2003). In contrast to the defects in growth of petal, stamen and style, the sepals of lip1, lip2 double mutants are larger than the wild type and have glands typical of bracts and leaves suggesting that lip1 and lip2 share a function in repressing some vegetative aspects that in a sepal context lead to increased organ size. Indeed the lip, lip2, def1 and the lip, lip2, ple triple mutants show that the organ effects are specific of each organ identity.

In terms of floral size and organ expansion, there is a large body of evidence supporting a key role of gibberellic acid (GAs). Many mutants affected in GA synthesis have underdeveloped floral organs (Oliszewski et al., 2002). The genes involved in repression of GA signalling belong to the DELLLA family of proteins and a decrease in the function of the protein family progressively restores stamen development in the strong gibberellin synthesis mutant GA1 (Cheng et al., 2004). Recent work shows that the floral homeotic genes AP3, PI and AG are directly activated by GA during flower development and using inducible promoters Yu et al., have shown that the homeotic genes are downstream of GA signalling (Yu et al., 2004).

**Genes controlling polar organ growth**

Pioneering work in Antirrhinum led the foundation of our current understanding of leaf development with the identification of a genetic program controlling dorsal/ventral (adaxial/abaxial) symmetry in lateral plant organs (Waites et al., 1998; Waites and Hudson, 1995). The PHANTASTICA gene product is a Myb transcription factor expressed throughout leaf primordia that establishes organ polarity. PHANTASTICA has functions in floral
development that include promotion of petal lobe development (Waites and Hudson, 2001) (see Fig. 3B) where adaxial/abaxial identity of the tissue is established. Phenotypic and genetic analysis of the HANDELBARS gene of Antirrhinum suggest that dorsal ventral asymmetry in floral organs might share components of the dorsal ventral pathway from leaves. Further genes involved in this process include the YABBY family of transcription factors (Bowman et al., 2002) and HD-ZIP III (Class III homeobox/leucine zipper) like REVOLUTA, PHABULOSA and PHAVOLUTA (see Hay et al., for a recent review) (Hay et al., 2004). Although most of the work on genes controlling establishment of organ polarity have been done in characterization of the leaf, some of the genes belonging to the YABBY family have floral phenotypes related to the subject of this review. The loss of function of the FILAMENTOUS FLOWER (FIL) gene causes loss of sepals and petals and substitution of stamens by filaments (Sawa et al., 1999). The lack of organ number in fil has been interpreted to be caused by the ectopic expression of some of the floral organ identity genes (Chen et al., 1999), suggesting that YABBY genes play a role in controlling patterning not only in the leaf but also in the flower. The ectopic expression of FIL does not cause increased floral organ number (Siegfried et al., 1999), but YABBY genes may play a general role in organ size development since expression of YABBY3 from the KANADI promoter produces giant flowers (Yuval Eshed, personal communication). This result would be in agreement with the model proposed by Chen et al., that maintains that the establishment of a proximo-distal axis controlled by YABBY genes might be important for organ expansion. This hypothesis is also favoured in maize where the mutant rolled leaf has been found to be important for lateral outgrowth of the leaf (Juarez et al., 2004).

In Antirrhinum the GRAMINIFOLIA (GRAM) gene promotes lateral growth of the leaf and has a subtle floral phenotype (Golz et al., 2004). Genetic and molecular interactions between GRAM and the gene controlling C function STYLOSA (STY) (Motte et al., 1998; Navarro et al., 2004), show that GRAM is involved in whorl positioning in the flower redundantly with STY. STYLOSA encodes an ortholog of the Drosophila/yeast GRO/TUP1 corepressor and the Arabidopsis gene LEUNIG (Conner and Liu, 2000) and seems to be affected in hormone mediated processes that affect both leaf and floral organs (Cnops et al., 2004; Navarro, 2004). LEUNIG and AINTEGUMENTA have been found to play a role in repression of C function and activation of cell division that supports marginal tissue development (Liu et al., 2000). All together this complex networks of genetic interactions suggest that genes controlling proximo-distal or adaxial-abaxial development interact with other genes that are required to establish identity boundaries and cell proliferation in a genetic network that is not yet very well understood.

Organ proportions

The maintenance of floral proportions is of primary importance in plants since both autogamous and allogamous species require the right positioning of the sexual organs to ensure either self pollination or pollen dispersal and the perianth plays a vital role ensuring the microclimate of humidity and protection against environmental factors required for fertilization. The literature provides several examples showing that not all the organs of a plant respond equally to disruption or modification of the cell cycle with the cell cycle machinery or cell expansion being affected depending on the cellular context. The expression of a CDC2 mutant in tobacco for example, which results in a cell cycle retardation, produces leaves of normal size with fewer, but bigger cells, whereas flowers and seeds contained fewer cells but of normal size. Whereas in the leaves the reduced proliferation was compensated by cell expansion, nuclear division cycle and cell expansion in flowers and seeds seemed to be more tightly coupled, (Hemerly et al., 1995). Similarly, ectopic expression of ANT, which results in bigger organs, causes an increase in cell division in the sepals, whereas the increased sizes in petals, stamens and carpels are primarily attributable to an elevated cell expansion (Krizek, 1999). Another example is the OVATE gene that represses growth in tomato fruits (Liu et al., 2002), since additional copies of the functional gene lead to a decrease in overall aerial growth including growth of leaves and floral organs. However sepals and stamens are more affected by the dosage effect of OVATE than petals and styles suggesting an interaction between the ovate gene product and the organ identity context.

We have been studying mutations specifically affected in floral proportions in Antirrhinum and the results show that at least perianth and sexual organ development, petal tube and petal lobes can be genetically separated in COMPACTA (Fig. 4A), COMPACTA ÄHNLICH (Fig. 4B) and UNILABIATA (Delgado-Benarroch, Weiss and Egea-Cortines unpublished results).

What limited data is available on this topic clearly suggests a differential growth response of each floral organ that is
intrinsic to its identity and that allows the proper floral proportions to be achieved, apparently by interaction of several developmental programs, some of them still to be identified.

**Control of organ size by cell division and expansion**

Mutant analysis and transformation experiments in plants show that changes in organ size can be traced back to an increase or decrease in either cell number or cell expansion or a combination of both, although changes in cell proliferation do not always correlate with changes in organ size because intrinsic mechanisms seem to coordinate cell proliferation and growth (Meyerowitz, 1997; Shpak *et al.*, 2003).

Cell division is regulated by the cell cycle machinery and a set of key transitions regulate the entry and progress through the cycle (G0-G1, G1-S and G2-mitosis). Transitions are controlled by the activity of cyclin-dependent protein kinases (CDKs) and these are typically activated by the synthesis of cyclins, other interacting proteins and the reversible phosphorylation of key amino-acid residues (Doonan, 2000). There are examples of a direct correlation between cell cycle and organ size variation (see examples above). On the other hand many works show that a variation in cell number does not cause variation in organ size. This is seen when *CDC2* is down regulated in transgenic tobacco plants, which produce normal sized leaves with less but larger cells (Hemerly *et al.*, 1995). Similarly, when cell division in young wheat leaves was blocked by gamma irradiation, leaf growth and morphogenesis continued and cell size increased compared to the non-irradiated control (Haber, 1962). Later studies showed that manipulation of cell division at the whole plant level might not modify organ size. However there are several reviews that explain these results (Meijer and Murray, 2001). Examples showing that cell division indeed plays a role in organ size include mutants with increased or decreased organ size attributable to changes in cell number. For instance in the mutant of *REVOLUTA/INTERFASCULAR FIBERLESS1* of *Arabidopsis*, growth and cell proliferation is prolonged, resulting in larger leaves and flowers and a bigger stem (Talbert *et al.*, 1995). On the other hand the *auxin resistant 1* mutant (*aux1*) has smaller leaves, inflorescence stems and floral organs caused by a decrease in cell number (Lincoln *et al.*, 1990). As the number of characterized mutants increases a general conclusion is that increases or decreases in organ size tend to be linked to modifications of the cell cycle, in terms of duration of proliferation, or to modifications in cell division capacity and potential.

Even though changes in cell expansion can be compensated to keep normal shape and size of an organ, some mutations that directly affect cell expansion show clear organ size phenotypes. The *Arabidopsis ROTUNDIFOLIA* (*ROT*) gene encodes a cytochrome P450 which appears to function specifically in polar elongation of leaf cells in the leaf-length direction (Kim *et al.*, 1998) and the *ANGUSTIFOLIA* (*AN*) gene which regulates polar elongation in the leaf-width direction (Folkers *et al.*, 2002; Kim *et al.*, 2002). The overexpression of *ROT3* in whole plant organs accelerates elongation of leaves and of floral organs derived from leaves, without affecting their width.

The emerging picture is that cell division is modulated at the organ level probably by signals that define the overall mass of the organ. One gene that seems to function allowing the leaf cells to arrest when needed is *CINNINNATA* (Nath *et al.*, 2003). The interesting aspect of *CIN* is that it links local cell division control to the much sought after systemic signal in leaf development and seems to be required to activate cell division in flowers (Crawford *et al.*, 2004). A detailed review about cell cycle genes can be seen in this issue (Ramirez-Parra *et al.*).

**Cell size and endoreduplication**

Plant growth happens via cell expansion, a complex process that has been recently reviewed (Martin *et al.*, 2001). Plants have two mechanisms to increase cell size beyond the regular size checkpoint that triggers cell division in eukaryotic cells (Coelho and Leewers, 2000) that might not be mutally exclusive. One is through the action of the vacuole, a specialized organ that allows cell expansion by increasing the intracellular volume without increasing the volume of cytoplasm. A second mechanism is endoreduplication, involving duplication of the genome without mitosis (Joubes and Chevalier, 2000). The increase in cell size correlates with an increased DNA content or ploidy level (Kondorosi *et al.*, 2000), so cell cycle progression and growth, which are normally coupled, are separated. It is thought that the bigger nuclei of polyploid cells meet the requirements of a higher metabolic activity, rRNA synthesis and transcriptional activity in larger cells. The tissue specific pattern of endopolyplid is characteristic of the species; in *Arabidopsis* it varies from 4C to 32C. In epidermal pavement cells of *Arabidopsis* morphometry reveals a direct proportionality between nuclear DNA level and cell size (Galbraith *et al.*, 1991). Endoreduplication does not happen in every cell as a mechanism to increase cell size, for instance *Arabidopsis* guard cells expand but maintain a 2C value (Melaragno *et al.*, 1993). Experiments where *Arabidopsis* replication licensing components have been overexpressed elegantly demonstrate that increased cell DNA content happens only in certain cell types (Castellano *et al.*, 2004). Interestingly, plants overexpressing *CDC8* show DNA content phenotypes but do not differ from wild type in terms of body size, suggesting that a general compensatory mechanism is exerted over the growth of the plant. Concerning flower tissues, *Arabidopsis* petals seem to be formed by cells that are largely 2C and endoreduplication has not been observed but in other members of the family, like cabbage, endoreduplication seems to be a common mechanism of petal cell size control (Kudo and Kimura, 2002).

Several mutations affecting cell wall formation in a general way have pleiotropic effects that suggest cell expansion is important to reach fully functional organs. For instance mutations like *ECTOPIC LIGNIFICATION 1* (*ELI1*) where cell expansion and lignification are impaired show pleiotropic phenotypes including decreased floral organ size (Caño-Delgado *et al.*, 2000). The mutant *FRAGILE FIBERS 2* (*FRA2*) is affected in cellulose fiber length and width results in a pleiotropic phenotype consisting of shorter but wider leaves and flowers (Burk *et al.*, 2001, Burk and Ye, 2002). These mutations clearly show that the process of cell wall deposition and formation plays an important role achieving the normal size and proportions of all plant organs. The expansins control cell wall loosening that is important for cell expansion (Cosgrove, 2000). Downregulation of an α-expansin in *Petunia hybrida* preferentially expressed in the petal limb causes a strong reduction in petal limb development whereas the floral tube.
formed by the fused part of the petals where the gene is not highly expressed remains normal in the transgenic plants (Zenoni et al., 2004). The phenotypic effects i.e. reduced expansion of the petal limbs (that are called lips in Antirrhinum) seems to be due to a decrease in cell expansion in both adaxial and abaxial epidermis. The results of Zenoni et al., suggest that differential growth might be achieved by specific gene expression pattern that give specificity of action to some genes, in this case involved in cell expansion. But it also illustrates that differential gene expression might be the cause of developmental compartments in floral organ development.

Conclusions

Most of the mendelian genes described in this review affect floral organ size together with other aspects of plant development, but the genetic analysis of some mutations and QTL in different plants show that in dicots, some of the regulatory networks controlling organ size might be specific for the flower. It will be interesting to see if the modularity is due to gene redundancy or to genes that are expressed only in the floral context. Some floral specific executors have been found for auxins (ARF and ANT) or gibberellins (AP3, PI and AG). We consider these important contributions because like other plant growth regulators they play general roles in development, but the important question is in the specific translation of their function in different tissues.

Nevertheless, new biological pathways will probably arise that are specific for the flower. A conceptual framework about the way cell division, expansion and growth direction are controlled to achieve organ growth and asymmetry has been laid down recently (Coen et al., 2004; Rolland-Lagan et al., 2003). It puts two important aspects in perspective, first the fact that organs might have specific compartments and second the existence of systemic signals that control rate of division, expansion and maybe more important for organ shape, the direction of these two events. Both in animals and plants there is a large body of evidence about this systemic signalling and we believe that one challenge in the future will be to identify mutations in that pathway that shed some light on this complex process.

Acknowledgements


