

Unusual positional effects on flower sex in an andromonoecious tree: Resource competition, architectural constraints, or inhibition by the apical flower?¹

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PREMISE OF THE STUDY: Two, nonmutually exclusive, mechanisms—competition for resources and architectural constraints—have been proposed to explain the proximal to distal decline in flower size, mass, and/or femaleness in indeterminate, elongate inflorescences. Whether these mechanisms also explain unusual positional effects such as distal to proximal declines of floral performance in determinate inflorescences, is understudied.

METHODS: We tested the relative influence of these mechanisms in the andromonoecious wild olive tree, where hermaphroditic flowers occur mainly on apical and the most proximal positions in determinate inflorescences. We experimentally increased the availability of resources for the inflorescences by removing half of the inflorescences per twig or reduced resource availability by removing leaves. We also removed the apical flower to test its inhibitory effect on subapical flowers.

KEY RESULTS: The apical flower had the highest probability of being hermaphroditic. Further down, however, the probability of finding a hermaphroditic flower decreased from the base to the tip of the inflorescences. An experimental increase of resources increased the probability of finding hermaphroditic flowers at each position, and vice versa. Removal of the apical flower increased the probability of producing hermaphroditic flowers in proximal positions but not in subapical positions.

CONCLUSIONS: These results indicate an interaction between resource competition and architectural constraints in influencing the arrangement of the hermaphroditic and male flowers within the inflorescences of the wild olive tree. Subapical flowers did not seem to be hormonally suppressed by apical flowers. The study of these unusual positional effects is needed for a general understanding about the functional implications of inflorescence architecture.

KEY WORDS andromonoecy; determinate inflorescences; flower sex; *Olea europaea* var. *sylvestris*; Oleaceae; positional effects; source–sink; wild olive

Within inflorescences, flowers may differ in shape, size, or function (e.g., sex) (Diggle, 1997, 2003; Ishii and Sakai, 2001; Miller and Diggle, 2003). Often, this intra-inflorescence variation follows a clear positional effect (Stephenson, 1981; Diggle, 1997). The most frequent positional effect within indeterminate elongate inflorescences of hermaphroditic species is a proximal to distal decline in flower size, corolla size, petal number, ovary size, ovule number, stamen number, and/or number of pollen grains (Diggle, 2003).

Two mechanisms have been proposed for positional effects. First, they may reflect a plastic response to intra-inflorescence competition for limited resources (Lloyd, 1980; Stephenson, 1981). Second, inherent architectural constraints in the design of inflorescences (Wolfe, 1992; Diggle, 1995, 2003) can influence flower size or reproductive performance. The relative importance of these two mechanisms differs among species (Diggle, 1997, 2003; Cao et al., 2011) and reproductive traits (Wolfe, 1992; Ortiz et al., 2009). Nevertheless, these two mechanisms are not mutually exclusive: inflorescence architecture often dictates how resources are allocated to proximal compared with distal flowers, by mechanical limitations in support structures, or in the vascular tissue that supplies resources (Wolfe, 1992; Wolfe and Denton, 2001) and/or by influencing the deployment order of flowers (Ishii and Sakai, 2001, 2002).

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A note of caution is needed concerning the universality of proximal–distal positional effects. For example, positional effects also exist in inflorescences with nonlinear architectures such as capitula (Torices and Méndez, 2010), umbels (Medrano et al., 2000), or cymes (Buide, 2008). More interestingly, some evidence suggests that determinate inflorescences have unusual positional effects that depart from the proximal–distal pattern. A distal–proximal pattern was described for *Iris fulva*, a plant having a determinate monochasial cyme (Wesselingh and Arnold, 2003). Distal–proximal patterns may appear if distal meristems represent stronger sinks for resources than proximal ones or produce hormones that form gradients along the inflorescence (Wesselingh and Arnold, 2003). In addition, in plants with basipetal anthesis, early fruit formation in distal positions may deplete available resources for proximal positions (Thomson, 1989). Inflorescences with unusual positional effects could shed light on how resource competition and architectural constraints interact to influence flower size, shape, or function because they show a mismatch between architecture and flower deployment (Wesselingh and Arnold, 2003).

As a consequence of positional effects, the emphasis in male or female functions in hermaphroditic plants may change dramatically within inflorescences (Diggle, 2003). Sex allocation theory (Charnov, 1982) predicts that resources allocated to male or female functions in a certain flower change in response to resource availability (Charlesworth and Charlesworth, 1981; Brunet and Charlesworth, 1995). Thus, if resources decline from proximal to distal positions, flowers should become progressively more male-biased upward in the inflorescences, as often observed (Mazer and Dawson, 2001; Ishii and Sakai, 2002).

Surprisingly, very little attention has been paid to positional effects in inflorescences of species with sexual systems such as monoecy, andromonoecy, or gynomonoecy, where male and/or female functions are segregated in separate flowers (but see Waller, 1988; Waller and Steingraeber, 1995; Miller and Diggle, 2003; Diggle and Miller, 2004). Causes of positional effects on flower sex in these species are key to understanding the evolution away from hermaphroditism. In particular, positional effects in andro-, gyno-, and monoecious plants are suggested to be originally resource-mediated positional effects in hermaphroditic ancestors that have, over evolutionary time, become architectural positional effects (Torices and Méndez, 2010). Evidence for this transition exists for andromonoecious *Solanum*, where a decrease in female allocation toward distal positions within an inflorescence has been found, e.g., proximal hermaphroditic and distal male flowers (Diggle and Miller, 2004). When fruit formation is unlikely in some positions as a consequence of a chronic lack of resources, optimal resource allocation could permanently switch off the female function in those flowers and lead to the evolution of andromonoecy (Primack and Lloyd, 1980; Emms, 1993; Brunet and Charlesworth, 1995). Accordingly, a loss of plasticity in flower sex is found along the phylogenetic tree of *Solanum* (Diggle and Miller, 2013). Testing the effect of resource manipulation in andromonoecious species with determinate inflorescences can help to establish the generality of this theoretical framework.

The wild olive tree (*Olea europaea* L. var. *sylvestris* (Miller) Lehr.; Oleaceae) is a suitable species for this purpose, because it is andromonoecious with determinate, cymose-paniculate inflorescences (Green, 2002) (Fig. 1A) showing an unusual positional pattern of flower sex. All flowers in this species are potentially hermaphroditic, and male flowers result from pistil abortion (Uriu, 1959). The

apical flower in *O. europaea* inflorescences is frequently hermaphroditic, whereas subapical flowers are mainly male (Cuevas and Polito, 2004; Seifi et al., 2008). The probability of finding a hermaphroditic flower in positions farther down the inflorescence is variable, but always lower than for the apical position and higher than for the subapical position (Seifi et al., 2008). Competition between flowers has been suggested to cause this positional effect (Seifi et al., 2008). However, the mechanisms underlying the preferential co-option of resources by the apical flower—hormonal inhibition vs. sink strength—remain unclear. If resource competition is the main cause of positional effects in this species, all inflorescence positions should show similar responses to resource manipulation (Fig. 1B). Alternatively, stronger architectural constraints would be revealed for those positions showing a lower response to resource manipulation (Fig. 1B).

Here, we report an experimental study that addresses how resource availability and the putatively inhibitory effect of the terminal flower influence the occurrence of flower sexes within the inflorescences of the wild olive tree. We first experimentally modified resource availability by removing either leaves or inflorescences from flowering twigs. These manipulations of resources should decrease (leaf removal) or increase (inflorescence removal) the probability of finding a hermaphroditic flower in any given position compared with control twigs (Fig. 1B). We then investigated whether the removal of the apical flower in the inflorescence increased the probability of producing hermaphroditic flowers in subapical positions (Fig. 1B).

MATERIALS AND METHODS

Study species and site—The wild olive tree, *Olea europaea* L. var. *sylvestris* (Miller) Lehr. (Oleaceae), is an evergreen species widespread in the Mediterranean basin (Rubio de Casas et al., 2006). It is andromonoecious (Green, 2002), i.e., bears both male and hermaphroditic flowers on the same individual. Both types of flowers can be found within the same inflorescence. Male flowers result from pistil abortion at varying stages of its development (Uriu, 1959). The most critical period for pistil abortion in *Olea* is about 1 month before full bloom (Uriu, 1959; Seifi et al., 2008). Flowers are borne on determinate inflorescences (Green, 2002). Twigs bear decussate leaves with two inflorescences per node, borne in the axils of the previous season leaves (Uriu, 1959). All inflorescences present a central axis terminated by a single, apical flower. Down this axis, a variable number of pairs of opposite flowers is arranged (Fig. 1A). Usually, in inflorescences with 11 or more flowers, there are secondary lateral axes at the base of the inflorescence (Fig. 2). As in the main axis, secondary axes end in a terminal flower, and flowers below are pairs of opposite flowers (Fig. 1A). The number of flowers and secondary axes per inflorescence (types: Fig. 2) is variable within individuals (Table 1). Number of flowers per inflorescence is dependent on twig vigor and position within the twig (Lavee et al., 1996, 1999). Both inflorescences in a given twig node, however, are of the same type. Flowering sequence within an inflorescence is bidirectional (Cuevas and Polito, 2004; Seifi et al., 2008) and proceeds from the middle toward both proximal and terminal positions. Terminal flowers on a secondary axis open first, followed by the apical flower, followed by flowers down along the central axis and other terminal flowers on secondary axes. Subapical flowers and lateral flowers on the secondary axes open last (Fig. 1A).

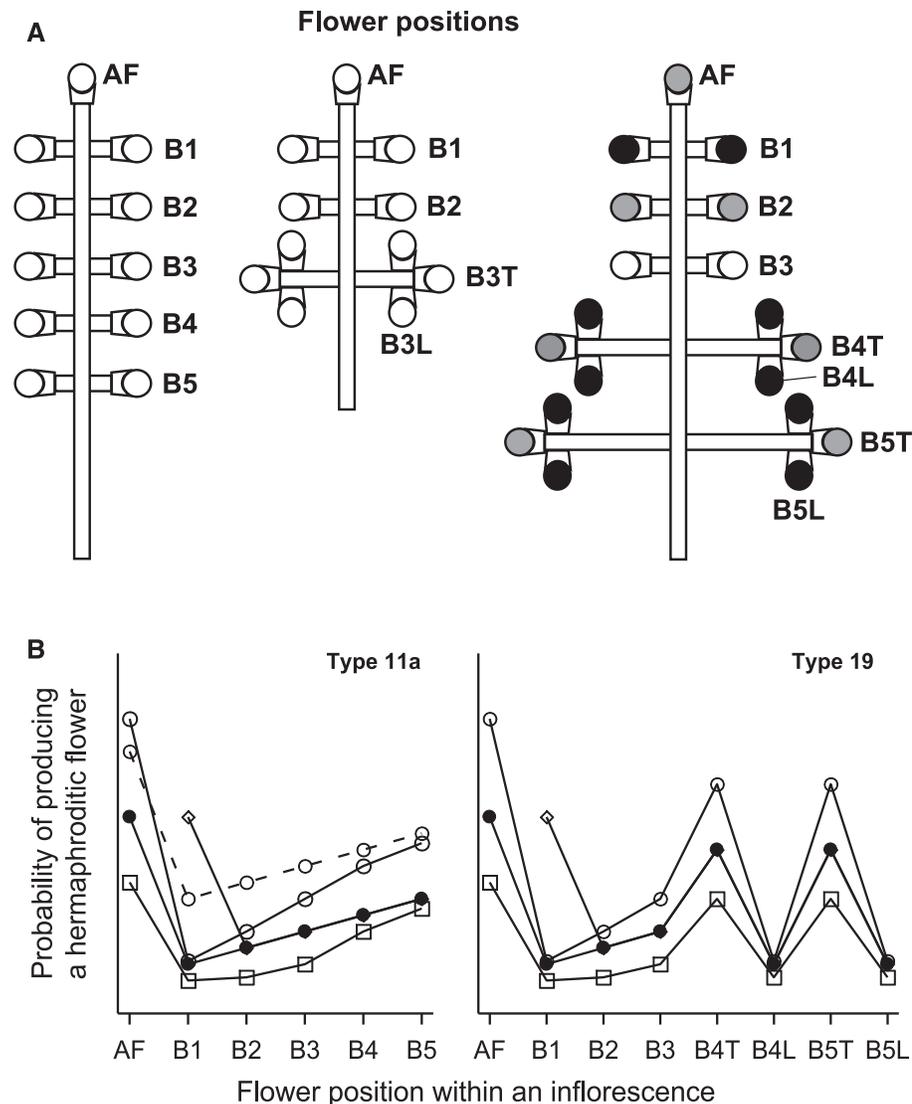


FIGURE 1 (A) Schematic representation of flower position within inflorescences without and with secondary axes in *Olea europaea* (modified from Seifi et al., 2008). AF = apical flower; B = branch; T = terminal; L = lateral. For inflorescences with 19 flowers, the order of blooming is indicated according to Seifi et al. (2008): earliest (white), middle (gray), latest (black). (B) Schedule of the probability of finding a hermaphroditic flower in different positions of control (black circle) inflorescences with or without secondary axes (see Fig. 2 for drawings of the types selected), compared with the expectations in the different experimental treatments. Inflorescence removal (open circles) is expected to increase the probability of a hermaphroditic flower, either similarly in all positions (dashed line in the left panel), indicating a main role for resource competition, or disproportionately for some positions, indicating stronger architectural constraints in the less-responsive positions (continuous line in the left panel). Leaf removal (open squares) is expected to decrease the probability of a hermaphroditic flower (only the disproportionate decrease is pictured). Removal of the apical flower (open diamond) is expected to increase the probability of finding a hermaphroditic flower in the subapical positions only if a hormonal inhibition is acting. The y-axes have no labels or ticks because these panels are intended to show qualitative trends only.

The current study was carried out in Aldea del Fresno, central Spain (40°20'N, 4°14'W, 500 m a.s.l.), in April–May 2008 and 2009. At this site, annual accumulated rainfall is 515 mm, and annual mean temperature is 15.8°C. The blooming peak of the wild olive at the study site typically occurs in mid-May.

Manipulation of twigs and inflorescences—

We selected 10 (5 in 2008 and 5 in 2009) similar-sized trees, ranging 2.5 to 3.5 m in height and 5 to 6.5 m in longest crown diameter, with high flowering intensity. On each tree, 40 to 52 flowering twigs were haphazardly chosen from the south side of the crown and at 1.5–2.0 m above the ground. We selected twigs separated from each other by at least two branching orders, lacking lateral vegetative growth, and having at least two-thirds of nodes with inflorescences. Flowering nodes per twig ranged from 3 to 8 (Table 1), with average values per tree ranging from 4.9 to 6.3 (Table 1). We ensured that vegetative nodes were not intercalated between flowering nodes. Labeling and manipulation of the twigs was carried out at the end of April, before pistils were likely to have aborted (Uriu, 1959; Seifi et al., 2008). At that time, inflorescences had started to swell, and it was possible to identify the branching pattern and number of flowers of the inflorescences. On each tree, 10 to 13 twigs were allocated to one of four experimental treatments: (1) removal of one inflorescence per node, alternating sides in the decussate arrangement (RemInf), (2) removal of one leaf per node, alternating sides in the decussate arrangement (RemLeaves), (3) removal with tweezers of the apical flower and part of its pedicel on all the inflorescences on the twig (RemAp), (4) no manipulation (Control). RemInf and RemLeaves were intended to increase and decrease, respectively, the resources available for inflorescences and thus induce a higher and lower, respectively, probability of producing hermaphroditic flowers at all positions of the inflorescences than in control inflorescences (Fig. 1B). We expected that these increases (decreases) would be either similar at all positions or they would interact with inflorescence architecture and be highest for apical and the most proximal positions (Fig. 1B). RemAp was intended to remove any potential hormonal inhibition effect of the apical flower and induce the production of hermaphroditic flowers in subapical positions within each inflorescence (Fig. 1B). To minimize damage to the twigs, we removed leaves and inflorescences using a mini leaf pruner and by cutting the petiole and peduncle, respectively, but leaving a short section attached to the stem, which eventually abscised.

Shortly before anthesis, in mid-May 2008 and 2009, we harvested the labeled twigs. We scored the branching pattern, number of flowers per inflorescence and sex of each flower at each position in the inflorescence. Flower position within the inflorescences was categorized following the method of Seifi et al. (2008) (Fig. 1A).

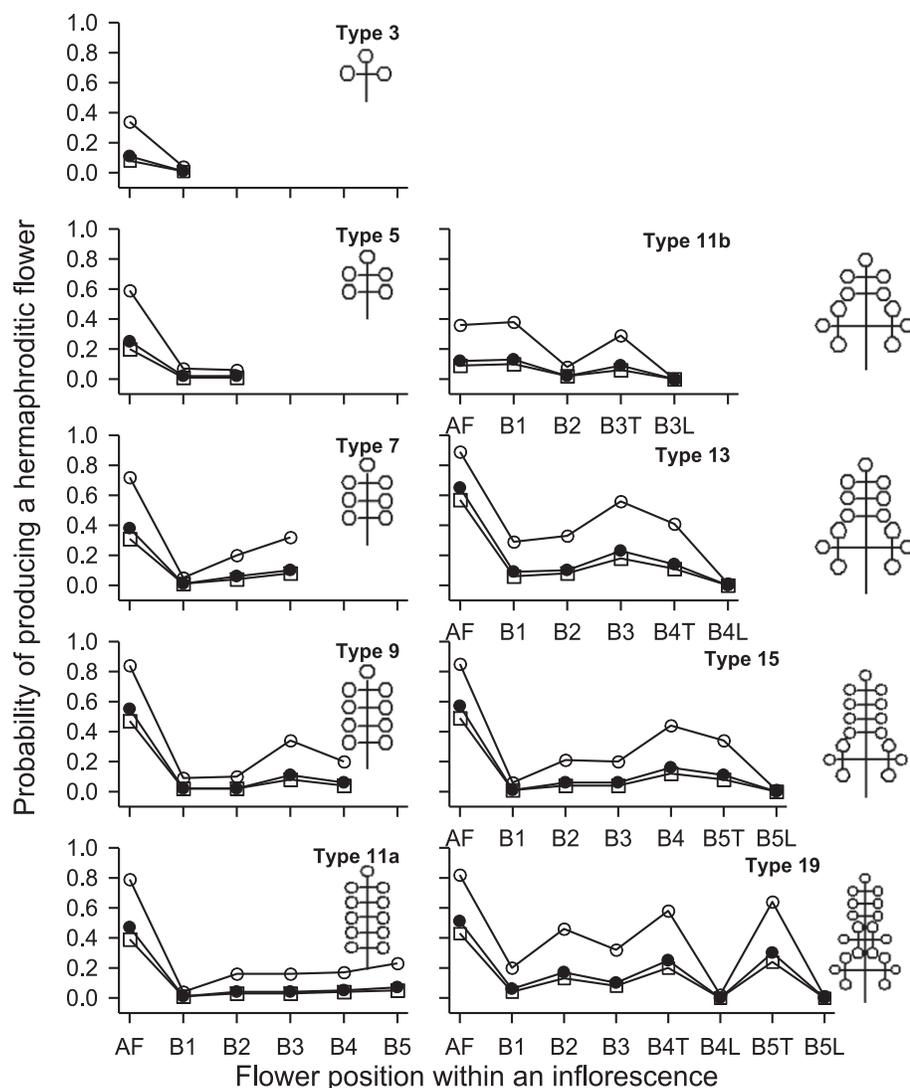


FIGURE 2 Results of the GLMMs testing the effect on flower sex of flower position within the inflorescence in control (closed circles) twigs and of experimental manipulations of inflorescence removal (open circles) and leaf removal (squares). Numbers are the predicted probabilities (back-transformed from the logit scale) of finding a hermaphroditic flower in that position (Appendix S1). Insets show the architecture of each inflorescence type. See Fig. 1 for the coding of flower positions and Appendix S3 for sample sizes.

Data analyses—To investigate how increasing or decreasing available resources and removal of the apical flower influenced flower sex at each position on the inflorescences, we used generalized linear mixed-effects models (GLMM) with a binomial error distribution and a logit-link function. GLMMs are appropriate to analyze hierarchically organized data (Pinheiro and Bates, 2000) as is the case of the arrangement of flowers within inflorescences, inflorescences within nodes and twigs, and twigs within plants. The Akaike information criterion (AIC) was used to select among alternative models (Burnham and Anderson, 2002). We chose the model with the lowest AIC among all models specified. The delta AIC (Δ_{AIC}), was then calculated as a measure of each model relative to the chosen model. Models differing in >2 units from the one with the lowest AIC had virtually no support and could be omitted. We followed a two-step procedure for model selection (Zuur et al., 2009). First,

we fit the beyond-optimum model for the fixed effects (i.e., the most complex model for the fixed effects, including their interactions) and compared models that differed in their random structure, namely: (1) no random factor; (2) the effect of plant on the intercept; (3) the effect of plant, and twig nested within plant on the intercept; and finally (3) the effect of plant, twig nested within plant, and node nested within twig on the intercept. Once the optimal random structure was found, we fit a set of models with a different structure in the fixed component, by generating all possible submodels from the most complex fixed effects structure. To test the effect of increasing or decreasing available resources on flower sex at each position on the inflorescences, the most complex model structure included Sex as a response variable, Pos Type, Treatment, and Year as fixed factors and all their interactions, as well as NodeId, Twig, and Tree as random factors. Sex is a binary variable specifying sex (1 = hermaphroditic, 0 = male); Pos Type is a factor that indicates flower position in each inflorescence type (Fig. 1); Treatment is a factor with three levels (RemInf, RemLeaves and Control); Year is a factor with two levels (2008, 2009); and NodeId, Twig and Tree are three random factors specifying the effect of the node where each inflorescence was sampled, nested within the twig, nested within the plant.

To assess whether removing the apical flower influenced the sex of the flowers at positions down in the inflorescence, the most complex model structure included Sex as a response variable, Pos Type, Apical, and Year as fixed factors and all their interactions, as well as NodeId, Twig, and Tree as random factors. Apical is a two-level factor specifying whether the inflorescence had the apical flower or not (data from Control and RemAp treatments, respectively), and the remaining terms are as described above.

All the analyses were performed using the lme4 package (Bates et al., 2015) in R (R Core Team, 2013).

RESULTS

Basic description of inflorescence architecture and positional effects—The number of flowers per inflorescence ranged from 1 to 29 (mean \pm SD: 10.0 ± 3.7). Approximately 97% of the inflorescences were classified in nine types based on their number of flowers and secondary axes (Fig. 2 and Table 1). Frequency of these nine types ranged from 1.4% (type 11a) to 36.8% (type 9; Table 1). Other types of inflorescences presented a frequency lower than 1% and were pooled as “others” in Table 1. Inflorescences with tertiary branches

TABLE 1. Number of sampled inflorescences per individual and descriptive statistics for the number of flowering nodes (mean and range) and percentage of hermaphroditic flowers (mean \pm SE). Inflorescences are segregated by their number of flowers (type) (see Fig. 2 for a graphical description of these types).

Year	Individual	No. flowering nodes	% Hermaphroditic flowers	No. inflorescences										Total
				Type 3	Type 5	Type 7	Type 9	Type 11a	Type 11b	Type 13	Type 15	Type 19	Others	
2008	1	5.4 (3–8)	75.3 \pm 1.3	2	0	6	9	2	2	19	17	45	30	132
	2	5.0 (3–7)	27.6 \pm 1.1	3	7	41	73	10	7	48	12	6	4	211
	3	5.8 (3–8)	8.7 \pm 0.6	0	10	23	140	0	8	7	7	3	0	198
	4	5.3 (3–7)	30.7 \pm 2.0	4	6	4	18	1	20	8	18	1	5	85
	5	5.8 (3–8)	1.8 \pm 0.4	6	2	22	69	1	33	9	31	7	10	190
2009	6	5.3 (3–8)	8.7 \pm 0.8	4	4	13	56	9	7	68	23	37	11	232
	7	6.3 (3–8)	15.3 \pm 0.7	4	6	38	161	3	13	78	14	23	4	344
	8	4.9 (3–8)	26.1 \pm 1.3	8	41	117	39	1	0	8	0	0	3	217
	9	5.8 (3–8)	18.7 \pm 1.0	0	1	126	93	3	3	72	1	1	0	300
	10	6.2 (3–8)	2.8 \pm 0.4	15	18	82	158	1	7	23	1	2	1	308
				46	95	472	816	31	100	340	124	125	68	2217

were not recorded within the most frequent types of inflorescences (Table 1).

The mean percentage of hermaphroditic flowers per tree ranged from 1.8 to 75.3% (Table 1). In control inflorescences, flower sex was influenced by the position of the flower within the inflorescence and the number of flowers per inflorescence (Fig. 2). In all types of control inflorescences, except for type 11b, the apical flower had the highest probability of being hermaphroditic (Fig. 2). In general, as the number of flowers per inflorescence increased, so did the probability of finding a hermaphroditic flower in the apical position, except for type 11b and 19 inflorescences (Fig. 2). Aside from apical flowers, the frequency of hermaphroditic flowers increased toward the base in most types of inflorescences (Fig. 2). Terminal flowers on secondary axes also had a fairly high (>10%) probability of being hermaphroditic, except in type 13 inflorescences (Fig. 2). A greater number of flowers per inflorescence, however, did not involve an increase in the proportion of hermaphroditic flowers per inflorescence.

Influence of resource manipulations on floral sex in different positions of the inflorescences—The best-fit model included the additive effect of flower position and treatment, but not year, as fixed terms, and the three nested random effects (Appendix S1, see Supplemental Data with this article). Compared with the control, removal of half of the inflorescences (RemInf) increased the probability of producing hermaphroditic flowers in most positions for all types of inflorescence (Fig. 2), while removal of half of the leaves (RemLeaves) in the twigs had the opposite effect (Fig. 2). Effects of RemInf treatment, however, were stronger than those of RemLeaves treatment (Fig. 2). The effect of the manipulation of resources was additive compared with the control (Appendix S1) and larger for the apical position, as well as for proximal flower positions and for terminal flowers on secondary axes (Fig. 2).

Influence of the apical flower on floral sex in different positions of the inflorescences—The best-fit model included the additive effect of flower position and apical flower removal, but not year, as fixed terms, and the three nested random effects (Appendix S2). Compared with the control inflorescences, the removal of the apical flower did not increase the probability of finding a hermaphroditic flower in the subapical position for most inflorescence types (Fig. 3). The effect of the RemAp treatment was additive to the model

(online Appendix S2) and involved a slight increase in the probabilities of being hermaphroditic for proximal, as well as for terminal flowers on secondary axes, compared with the control (Fig. 3).

DISCUSSION

Our results suggest a role for both resource competition and architectural constraints in the unusual positional effects within the inflorescences of *Olea europaea*. When more resources were available due to inflorescence removal, flower positions other than the apical ones increased their probability of producing a hermaphroditic flower. This result indicates that competition for limited resources was underlying sex expression at different positions within the inflorescence. Nevertheless, this increase in the probability of producing a hermaphroditic flower still was stronger for apical, compared with other flower positions along the inflorescence, suggesting that certain positions in the inflorescences are under stronger architectural constraints. Removal of the apical flower did not release subapical positions from producing mainly male flowers. Thus, the inhibitory effect of the apical flower did not seem to be based on hormonal control. Below, we discuss the implications of these findings for *Olea* and, more generally, the functional implications of inflorescence architecture.

Influence of resource manipulations on floral sex in different positions of the inflorescences—Removal of leaves or inflorescences in the flowering twigs affected the proportion of hermaphroditic flowers. The effects of flower removal were in line with the findings of the few resource manipulation studies in other andromonoecious species with plastic sexual allocation (Emms, 1993; Diggle, 1994). The leaf removal treatment barely affected the probability of producing hermaphroditic flowers in most inflorescence positions (see Lovett Doust and Harper, 1980 and Parra-Tabla et al., 2004 for similar results). There are two potential explanations for this result. First, leaf removal was not severe enough to affect pistil abortion. Second, the resources allocated to inflorescences and flowers may come from plant storage rather than from current photoassimilate (Lavee et al., 1996, 1999). Inflorescence removal was expected to increase resource availability and thus the proportion of hermaphroditic flowers per inflorescence (Krupnick and Weis, 1998), while leaf removal should have the opposite effects

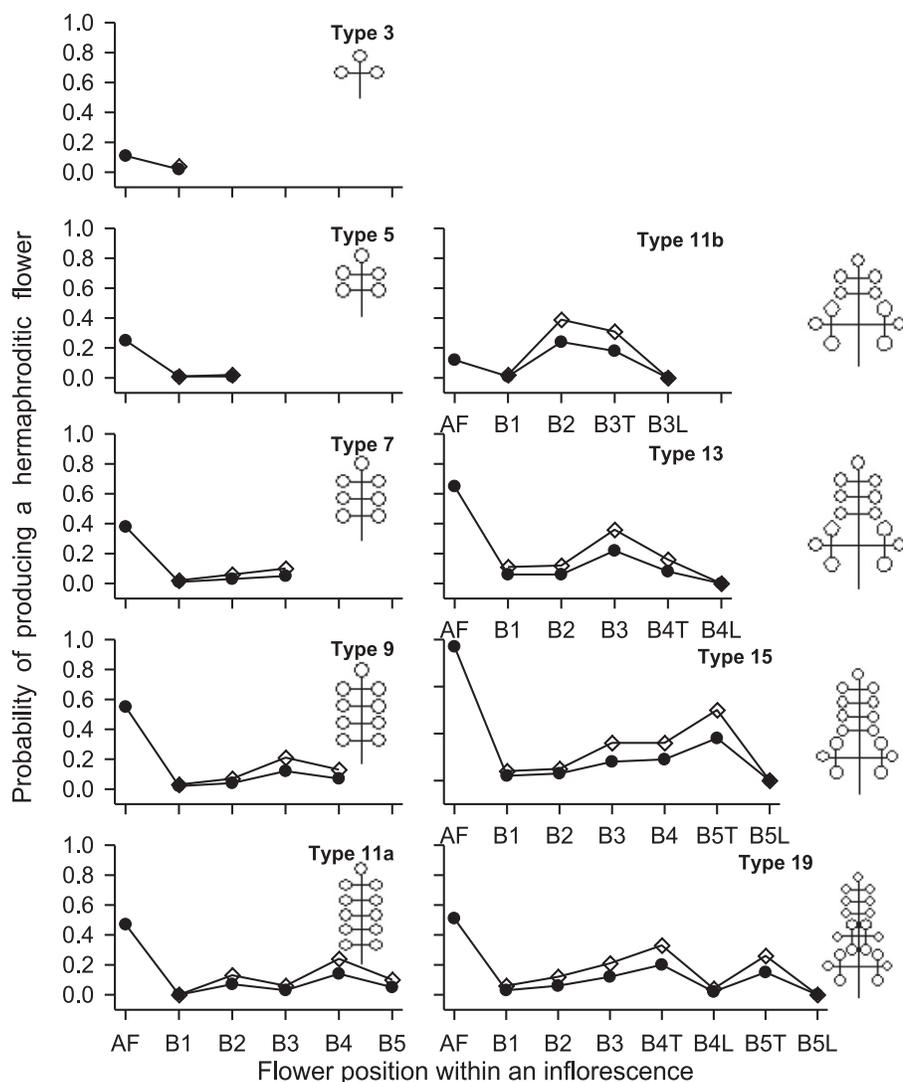


FIGURE 3 Results of the GLMMs testing the effect on flower sex of flower position within the inflorescence in control (closed circles) twigs, and of experimental manipulations of apical flower removal (diamonds). Numbers are the predicted probabilities (back-transformed from the logit scale) of finding a hermaphroditic flower in that position (Appendix S2). Insets show the architecture of each inflorescence type. See Fig. 1 for the coding of flower positions and Appendix S3 for sample sizes.

(Spears and May, 1988). In this case, competition between inflorescences seemed to be more influential than the number of leaves supplying resources. This finding suggests that resource partitioning among flowers in wild olive trees is sink-regulated, rather than source (Ho, 1992).

The change in the proportion of hermaphrodite flowers in response to the experimental treatments was not the same in all positions of the inflorescence. Instead, such change compared with the control was position dependent, and its magnitude was larger in those positions that produced hermaphroditic flowers more frequently, i.e., the terminal flowers on the lateral branches and, to a lesser degree, proximal positions (Fig. 1B). Terminal flower positions still retained their dominance and co-opted a disproportionate amount of the extra resources, indicating that pure architectural effects were also involved.

The joint evolution of andromonoecy and positional effects on flower sex is supposed to derive from a loss of the female function in flowers located at positions where seed production is low (Primack and Lloyd, 1980; Brunet and Charlesworth, 1995; Waller and Steingraeber, 1995; Miller and Diggle, 2003). This idea is supported by evidence from andromonoecious species with plasticity in their positional effects, where inflorescence architecture can mediate sexual expression (Miller and Diggle, 2003). Two research lines can provide useful insights here. First, intraspecific variation should be explored. Variation in wild plants and among cultivars in the average number of flowers per inflorescence and in the number of secondary axes (preferential positions for hermaphroditic flowers) (Dimassi et al., 1999; Seifi et al., 2008) opens the possibility for a feedback between inflorescence architecture and sexual expression in *Olea europaea*. Evidence is mixed because production of male flowers increased with the number of total flowers per inflorescence in a comparison of three cultivars (Seifi et al., 2008), but not in a comparison of 15 cultivars (Dimassi et al., 1999). Consideration of inflorescence architecture in *Olea* cultivars in the light of optimal reproductive allocation deserves further study because it could have applied interest for improving yield under different environmental conditions. Second, interspecific variation should be examined. Position effects on fruit set in hermaphroditic species of *Olea* are expected to be similar to the position effects on floral sex in wild olive tree.

Influence of the apical flower on floral sex on different positions of the inflorescences—

Resource-mediated sink strength, rather than some hormonal inhibition of other flower meristems (Wesselingh and Arnold, 2003), seemed to be involved in the inhibitory effect of the apical flower. The removal of the apical flower did not “release” subapical flowers from pistil abortion, as would be expected from a hormone-mediated effect on flower sex. Instead, proximal flowers increased their probability of becoming hermaphroditic. This result is consistent with resource reallocation toward the floral positions closest to the resource supply, when a strong sink is removed. Strong sink strength of apical flowers has been demonstrated for rice grains (Mohapatra et al., 2011). The strong sink strength of the apical flower is also suggested by the fact that the probability of producing a hermaphroditic flower in the apical position was proportional to inflorescence size.

The mechanistic basis of resource co-option by the apical flower and the terminal flowers on secondary axes in *O. europaea* inflorescences is unknown. Although resource co-option by the apical flower is present, the otherwise proximal to distal decrease in frequency of hermaphroditic flowers suggests the action of some

architectural constraint that prioritizes the apical flower meristem, such as independent vascular bundles. In fact, resource partitioning along inflorescences of *O. europaea* shows complex patterns, with higher N, Mg, and Mn concentration at apical positions, but higher P, Fe, and Zn at proximal positions (Bouranis et al., 1999).

Can the production of a hermaphroditic flower in the apical position of the wild olive tree inflorescences be considered as adaptive or as an unescapable constraint of determinate inflorescence architecture (Waller and Steingraeber, 1995)? Adaptive reasons are expected if processes such as the ones suggested by Brunet and Charlesworth (1995) are occurring in the conversion of plastic positional effects into fixed architectural constraints. Two adaptive explanations are possible. First, the apical position is the most exposed position, so it may receive more outcross pollen (see Arista et al., 1999 for a similar reasoning). Second, fruit formation in the apical position may facilitate fruit removal by birds that would enhance seed dispersal. At present, both possibilities remain speculative for *O. europaea*. Mere architectural constraints could account for the presence of an apical hermaphroditic flower. Nevertheless, adaptive explanations deserve further scrutiny.

In conclusion, our study expands our knowledge about the ecological and evolutionary implications of inflorescence architecture by explicitly applying the traditional framework of positional effects within inflorescences to an andromonoecious species with determinate inflorescences. We experimentally showed that both intra-inflorescence competition for limited resources and architectural effects are involved in unusual positional effects, where flower position and flower deployment are not tightly coupled. Positional effects on flower sex are known for many other species with determinate inflorescences and andro-, gyno- or monoecious sexual systems (Endress, 2010; M. Méndez, unpublished data). The study of the proximate mechanisms and ultimate causes underlying these unusual positional effects is needed for a general understanding of the role that constraints and adaptive processes have played in shaping the diversity of inflorescence architectures and sexual systems.

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LITERATURE CITED

- Arista, M., P. L. Ortiz, and S. Talavera. 1999. Apical pattern of fruit production in the racemes of *Ceratonia siliqua* (Leguminosae: Caesalpinoideae): Role of pollinators. *American Journal of Botany* 86: 1708–1716.
- Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear-mixed-effects models using lme4. *Journal of Statistical Software* 67: 1–48.
- Bouranis, D. L., C. K. Kitsaki, S. N. Chorianopoulou, G. Aivalakis, and J. B. Drossopoulos. 1999. Nutritional dynamics of olive tree flowers. *Journal of Plant Nutrition* 22: 245–257.
- Brunet, J., and D. Charlesworth. 1995. Floral sex allocation in sequentially blooming plants. *Evolution* 49: 70–79.
- Buide, M. L. 2008. Disentangling the causes of intrainflorescence variation in floral traits and fecundity in the hermaphrodite *Silene acutifolia*. *American Journal of Botany* 95: 490–497.
- Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: A practical information-theoretic approach. Springer-Verlag, New York, New York, USA.
- Cao, G. X., L. Xue, Y. Li, and K. W. Pan. 2011. The relative importance of architecture and resource competition in allocation to pollen and ovule number within inflorescences of *Hosta ventricosa* varies with the resource pools. *Annals of Botany* 107: 1413–1419.
- Charlesworth, D., and B. Charlesworth. 1981. Allocation of resources to male and female functions in hermaphrodites. *Biological Journal of the Linnean Society* 15: 57–74.
- Charnov, E. L. 1982. The theory of sex allocation. Princeton University Press, Princeton, New Jersey, USA.
- Cuevas, J., and V. S. Polito. 2004. The role of staminate flowers in the breeding system of *Olea europaea* (Oleaceae): An andromonoecious, wind-pollinated taxon. *Annals of Botany* 93: 547–553.
- Diggle, P. K. 1994. The expression of andromonoecy in *Solanum hirtum* (Solanaceae): Phenotypic plasticity and ontogenetic contingency. *American Journal of Botany* 81: 1354–1365.
- Diggle, P. K. 1995. Architectural effects and the interpretation of patterns of fruit and seed development. *Annual Review of Ecology and Systematics* 26: 531–552.
- Diggle, P. K. 1997. Ontogenetic contingency and floral morphology: The effects of architecture and resource limitation. *International Journal of Plant Sciences* 158: S99–S107.
- Diggle, P. K. 2003. Architectural effects on floral form and function: A review. In T. Stuessy, V. Mayer, and E. Hörandl [eds.], *Deep morphology: Toward a renaissance of morphology in plant systematics*, 63–80. A. R. G. Gantner-Verlag, Ruggell, Liechtenstein.
- Diggle, P. K., and J. S. Miller. 2004. Architectural effects mimic floral sexual dimorphism in *Solanum* (Solanaceae). *American Journal of Botany* 91: 2030–2040.
- Diggle, P. K., and J. S. Miller. 2013. Developmental plasticity, genetic assimilation, and the evolutionary diversification of sexual expression in *Solanum*. *American Journal of Botany* 100: 1050–1060.
- Dimassi, K., I. Therios, and A. Balatsos. 1999. The blooming period and self-fruitfulness in twelve Greek and three foreign olive cultivars. *Acta Horticulturae* 474: 275–278.
- Emms, S. K. 1993. Andromonoecy in *Zigadenus paniculatus* (Liliaceae): Spatial and temporal patterns of sex allocation. *American Journal of Botany* 80: 914–923.
- Endress, P. K. 2010. Disentangling confusions in inflorescence morphology: Patterns and diversity of reproductive shoot ramification in angiosperms. *Bulletin of Systematics and Evolution* 48: 225–239.
- Green, P. S. 2002. A revision of *Olea* L. (Oleaceae). *Kew Bulletin* 57: 91–140.
- Ho, L. C. 1992. Fruit growth and sink strength. In C. Marshall and J. Grace [eds.], *Fruit and seed production: Aspects of development, environmental physiology and ecology*, 101–124. Cambridge University Press, Cambridge, UK.
- Ishii, H. S., and S. Sakai. 2001. Effects of display size and position on individual floral longevity in racemes of *Nartheicum asiaticum* (Liliaceae). *Functional Ecology* 15: 396–405.
- Ishii, H. S., and S. Sakai. 2002. Temporal variation in floral display size and individual floral sex allocation in racemes of *Nartheicum asiaticum* (Liliaceae). *American Journal of Botany* 89: 441–446.
- Krupnick, G. A., and A. E. Weis. 1998. Floral herbivore effect on the sex expression of an andromonoecious plant, *Isomeris arborea* (Capparaceae). *Plant Ecology* 134: 151–162.
- Lavee, S., L. Rallo, H. F. Rapoport, and A. Troncoso. 1996. The floral biology of the olive: Effect of flower number, type and distribution on fruitset. *Scientia Horticulturae* 66: 149–158.
- Lavee, S., L. Rallo, H. F. Rapoport, and A. Troncoso. 1999. The floral biology of the olive: II. The effect of inflorescence load and distribution per shoot on fruit set and load. *Scientia Horticulturae* 82: 181–192.

- Lloyd, D. G. 1980. Sexual strategies in plants. I. An hypothesis of sexual adjustment of maternal investment during one reproductive season. *New Phytologist* 86: 69–79.
- Lovett Doust, J., and J. L. Harper. 1980. The resource costs of gender and maternal support in an andromonoecious umbellifer, *Smyrniolum olusatrum* L. *New Phytologist* 85: 251–264.
- Mazer, S. J., and K. A. Dawson. 2001. Size-dependent sex allocation within flowers of the annual herb *Clarkia unguiculata* (Onagraceae): Ontogenetic and among-plant variation. *American Journal of Botany* 88: 819–831.
- Medrano, M., P. Guitián, and J. Guitián. 2000. Patterns of fruit and seed set within inflorescences of *Pancratium maritimum* (Amaryllidaceae): Nonuniform pollination, resource limitation, or architectural effects? *American Journal of Botany* 87: 493–501.
- Miller, J. S., and P. K. Diggle. 2003. Diversification of andromonoecy in *Solanum* section *Lasiocarpa* (Solanaceae): The roles of phenotypic plasticity and architecture. *American Journal of Botany* 90: 707–715.
- Mohapatra, P. K., R. Panigrahi, and N. C. Turner. 2011. Physiology of spikelet development on the rice panicle: Is manipulation of apical dominance crucial for grain yield improvement? *Advances in Agronomy* 110: 333–359.
- Ortiz, P. L., R. Berjano, M. Talavera, and M. Arista. 2009. The role of resources and architecture in modeling floral variability for the monoecious amphicarpic *Emex spinosa* (Polygonaceae). *American Journal of Botany* 96: 2062–2073.
- Parra-Tabla, V., V. Rico-Gray, and M. Carbajal. 2004. Effect of defoliation on leaf growth, sexual expression and reproductive success in *Cnidoscolus aconitifolius* (Euphorbiaceae). *Plant Ecology* 173: 153–160.
- Pinheiro, J. C., and D. M. Bates. 2000. Mixed effects models in S and S-Plus. Springer-Verlag, Berlin, Germany.
- Primack, R. B., and D. G. Lloyd. 1980. Andromonoecy in the New Zealand montane shrub Manuka, *Leptospermum scoparium* (Myrtaceae). *American Journal of Botany* 67: 361–368.
- R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Rubio de Casas, R., G. Besnard, P. Schönswetter, L. Balaguer, and P. Vargas. 2006. Extensive gene flow blurs phylogeographic but not phylogenetic signal in *Olea europaea* L. *Theoretical and Applied Genetics* 113: 575–583.
- Seifi, E., J. Guerin, B. Kaiser, and M. Sedgley. 2008. Inflorescence architecture of olive. *Scientia Horticulturae* 116: 273–279.
- Spears, E. E. Jr., and P. G. May. 1988. Effect of defoliation on gender expression and fruit set in *Passiflora incarnata*. *American Journal of Botany* 75: 1842–1847.
- Stephenson, A. G. 1981. Flower and fruit abortion: Proximate causes and ultimate functions. *Annual Review of Ecology and Systematics* 12: 253–279.
- Thomson, J. D. 1989. Deployment of ovules and pollen among flowers within inflorescences. *Evolutionary Trends in Plants* 3: 65–68.
- Torices, R., and M. Méndez. 2010. Fruit size decline from the margin to the center of capitula is the result of resource competition and architectural constraints. *Oecologia* 164: 949–958.
- Uriu, K. 1959. Periods of pistil abortion in the development of the olive flower. *Proceedings of the American Society for Horticultural Science* 73: 194–202.
- Waller, D. M. 1988. Plant morphology and reproduction. In J. Lovett-Doust and L. Lovett-Doust [eds.], *Plant reproductive ecology: Patterns and strategies*, 203–227. Oxford University Press, New York, New York, USA.
- Waller, D. M., and D. A. Steingraeber. 1995. Opportunities and constraints in the placement of flowers and fruits. In B. L. Gartner [ed.] *Plant stems: Physiology and functional morphology*, 51–73. Academic Press, San Diego, California, USA.
- Wesselingh, R. A., and M. L. Arnold. 2003. A top-down hierarchy in fruit set on inflorescences in *Iris fulva* (Iridaceae). *Plant Biology* 5: 651–660.
- Wolfe, L. M. 1992. Why does the size of reproductive structures decline through time in *Hydrophyllum appendiculatum* (Hydrophyllaceae)? Developmental constraints vs. resource limitation. *American Journal of Botany* 79: 1286–1290.
- Wolfe, L. M., and W. Denton. 2001. Morphological constraints on fruit size in *Linaria canadensis*. *International Journal of Plant Sciences* 162: 1313–1316.
- Zuur, A. F., E. N. Ieno, N. Walker, A. A. Saveliev, and G. M. Smith. 2009. Mixed effects models and extensions in ecology with R. Springer-Verlag, New York, New York, USA.