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Physiological and reproductive differences between hermaphrodites and males in the androdioecious plant *Fraxinus ornus*

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Androdioecy, the co-occurrence of males and hermaphrodites in a breeding population, is a rare reproductive system in the nature. This rarity may be the result of the large fitness gain required for male plants to be maintained by selection. Physiological, vegetative and reproductive characters of males and hermaphrodites of the androdioecious species *Fraxinus ornus* (Oleaceae) were compared, supporting the hypothesis that males compensate the fitness advantage of hermaphrodites with greater reproductive -but not vegetative- output, with concomitant differences in physiological capacities between the genders. Photosynthetic rate was similar between both genders, but hermaphrodites had lower water potential and carbon isotope discrimination than males. Photosynthesis rates decreased with decreasing water potentials more steeply in males than in hermaphrodites, indicating that hermaphrodites were more drought tolerant than males. Vegetative characters such as current year shoot growth or tree size did not differ between genders. Males produced 1.6 times more inflorescences than hermaphrodites. This difference was consistent across years and populations.

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Growing empirical evidence supports Lloyd and Webb's (1977) prediction that females have higher costs of reproduction than males (reviewed by Delph 1999 and Obeso 2002). A gender-differential investment in major plant fitness components (vegetative growth, reproduction, and defence against predators/herbivores) has usually been found, with females allocating more energy to reproduction and defence, and males more to vegetative growth (Putwain and Harper 1972, Boecklen et al. 1990).

Much less attention has been paid to the underlying physiological processes driving these ecological and morphological differences (Obeso et al. 1998, Dawson and Geber 1999, Correia and Diaz Barradas 2000, Retuerto et al. 2000). Physiology is a critical point because variation in physiological attributes may determine the performance of each sex in different microhabitats and, consequently, the gender-differential allocation to growth, reproduction and defence (Retuerto et al. 2000). Theoretically, sex differences in reproductive effort may increase physiological stress in the gender with the highest reproductive investment (Dawson and Ehleringer 1993), but this prediction remains untested at a general level because of the few studies available (Dawson and Geber 1999).

Gender differences associated with reproduction in sexually dimorphic plants allow comparisons between individuals allocating much energy to reproduction vs individuals allocating less (Obeso 2002). These comparisons have usually been made between female and male individuals from dioecious species (Delph 1999), although Obeso (2002) included in his review a small number of intersexual comparisons from non-dioecious species (sub-dioecious, gynodioecious and trioecious species), and a recent review has been made for gynodioecious species (Shykoff et al. 2003). However,

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intersexual comparisons in androdioecious species (males vs hermaphrodites) have rarely been made, although this reproductive system should provide the most asymmetric situation in terms of costs of reproduction (Sleeman et al. 2002). This asymmetry is produced if the costs of the female function are greater than those of male function, and therefore 1) males invest only in the cheap (male) function; 2) females invest only in the expensive (female) function and 3) hermaphrodites invest in both the cheap and the expensive functions. Thus, the continuum of reproduction costs will be males < females < hermaphrodites and the intersexual comparison under androdioecy represent the most asymetrical situation (two extremes of the continuum) whereas dioecy or gynodioecy represent less asymmetric comparisons (an extrem and the middle of the continuum).

The rarity of androdioecy in the nature has led both to the development of a theoretical corpus explaining its evolutionary maintenance (Lloyd 1975, Charlesworth 1984, Pannell and Ojeda 2000, Pannell 2001, 2002a) and to the search for new examples in nature (Liston et al. 1990, Aronne and Wilcock 1992, Lepart and Dommée 1992, Pannell 1997, Ishida and Hiura 1998, Akimoto et al. 1999, Dommée et al. 1999, Sakai 2001). In a functionally androdioecious species, males must sire more than twice as many successful offspring as do the hermaphrodites (Charlesworth 1984, Pannell 2002b). This has stimulated comparisons between males and hermaphrodites in terms of their pollen production (Lepart and Dommée 1992, Philbrick and Rieseberg 1994, Traveset 1994, Ishida and Hiura 1998, Vassiliadis et al. 2000), but little is yet known about possible different physiological capacities between the genders, which may affect their reproductive and vegetative allocation.

Similarly to the theoretical expectations for the maintenance of female individuals in gynodioecious populations, males in androdioecious populations must compensate for lost female functions expressed by hermaphrodites. Common forms of compensation are either vegetative outperformance, or higher fecundity of unisexuals relative to hermaphrodites (Alonso and Herrera 2001, Shykoff et al. 2003). It is hypothesized that such compensation is possible because of the resources made available by reduced female fertility, and this may be reflected in physiological differences between the genders.

The aim of this paper is to compare physiological, vegetative and reproductive characters of males and hermaphrodites of the androdioecious species *Fraxinus ornus* under the hypothesis that males compensate the fitness advantage of hermaphrodites with greater vegetative and/or reproductive output, with concomitant differences in physiological capacities between the genders. *Fraxinus ornus* is one of several species in the Oleaceae that display androdioecy (Dommée et al. 1999, Wallander 2001).

Methods

Study sites and species

Fraxinus ornus (Oleaceae) is a small deciduous tree that may reach up to 20 m. It inhabits mixed woods, thickets and rocky places in the Mediterranean region and Central Europe (Tutin et al. 1972). The only natural populations in Spain are located in the Valencian Community region (Fig. 1), representing the western limit of the distribution range. Leaves flush at the same time as flowers, in spring. The small white flowers, hermaphrodite or male, are presented in terminal inflorescences, and pollination occurs via both insects and wind (Wallander 2001). Fruits are samaras that ripen in autumn. Annual seed production is very irregular, as in other *Fraxinus* species (Tapper 1992, 1996).

I selected five study populations distributed along the altitudinal range of *Fraxinus ornus* in Spain (Fig. 1). All the populations are Mediterranean scrublands that experience a typical Mediterranean climate, with hot and dry summers and cold winters. The main part of this study was carried out in "La Font Roja" Natural Park, because this population is the most extensive and less disturbed population. Mean annual precipitation and temperature in this population are 825 mm and 11.6°C, respectively. More details of this study area can be found in Laguna and García (1988).

I estimated gender of flowering individuals in two populations (Alzira and Font Roja) in four consecutive years (1999–2002). None of the eight population gender ratios estimated (4 years × 2 populations) differed statistically from 1:1 (chi-square test). I estimated gender ratio by multiplying the number of individuals of each gender by the proportion of flowering individuals in each gender. Specifically, at the Alcira population, I recorded the gender of all the individuals (141 males and 132 hermaphrodites) found in a 900 × 8 m plot. The percentage of flowering individuals at the Alcira population was estimated every year from a sub-sample of 20 hermaphrodites and 21 males that had been tagged the first year. At Font Roja population, 128 individuals (67 males and 61 hermaphrodites) were sexed in a 1400 × 5 m plot and the percentage of flowering individuals was estimated every year from a subsample of 22 tagged hermaphrodites and 20 tagged males.

Fraxinus ornus is clearly morphologically androdioecious, but the functionality of the reproductive system is still under debate (Dommée et al. 1999, Wallander 2001, Pannell 2002a) since a 1:1 population gender ratio suggests functional dioecy (Charlesworth 1984, Pannell 2002a).

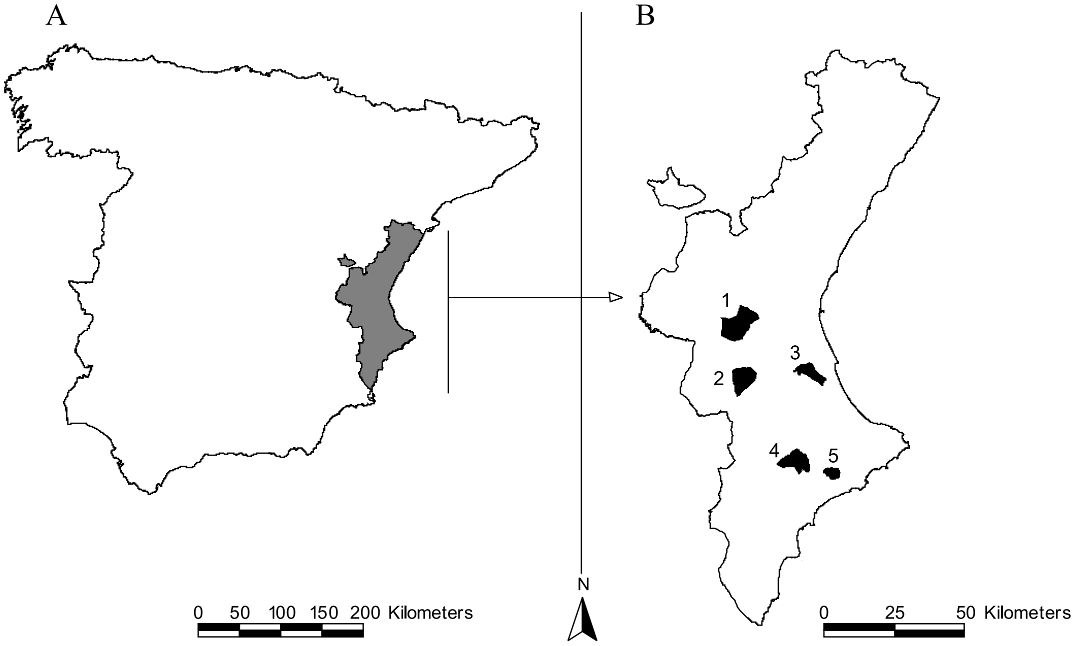

Fig. 1. (A) Map of Spain showing Valencian community. (B) Location and altitude (m) of the study populations. 1, Bunyol (800 m); 2, Bicorp (600 m); 3, Alzira (300 m); 4, Font Roja (1350 m); 5, Sella (450 m).

Physiological characters

I selected 10 males and 9 hermaphrodites of similar size in Font Roja to monitor their physiological status (photosynthesis rate and water potential) during the period when leaves were mature (from May to September 2002). Trees were selected so that the distance between a male and a hermaphrodite was minimized and thus the measures could be alternated between individuals of different gender. By doing this I avoided confounding gender effects with the time of day when measurements were taken.

I measured photosynthesic rate every month on cloudless days, between 8:00 and 10:00 GMT, with a portable photosynthesis infra-red gas analyzer (ADC-LCi). This is the time in which plants in Mediterranean communities have the maximum gas exchange capacity, which declines strongly at mid-day on summer days (Tenhunen et al. 1981, Lange et al. 1982). Photosynthesis was usually measured in one leaf per tree, maintaining its original position; therefore incoming light intensity could be very different between individual measurements. To account for this difference, the photosynthetic active radiation (PAR) was also recorded and used as a covariate in the statistical analysis. A repeated measures analysis of variance was run in the MIXED linear model procedure of SPSS 11.0.1 that expands the classical general linear model to allow the data to exhibit correlation and non-constant variability. This procedure not only provides more correct standard errors and appropriate tests of significance for fixed effects from unbalanced datasets than the GLM procedure, but also allows modelling the variance and covariance of correlated data resulting from repeated measures (Saavedra and Douglass 2002). An autoregressive of order 1 covariance structure was chosen because monthly physiological measures may be time-correlated (i.e. measures from two consecutive months should be more similar than measures from two distant months). PAR was included as a time-dependent covariate, gender was considered a fixed factor, and time (month) as the repeated factor.

After finishing the photosynthesis measurements, I recorded water potential in one shoot excised from each of the same trees. A digital Skye Plant Moisture System following the Scholander pressure bomb technique (SKPM-1400/80 bars) was used. The same statistical analysis (without covariate) was used to analyse the photosynthesis data.

I obtained carbon isotope discrimination as a time-integrative measure of plant physiological activity (Rundel et al. 1988), using leaves collected before senescence at the end of the season. Analyses were run in the Laboratorio de Isótopos Estables (Universidad Autónoma de Madrid, Spain) with a EA-CF-IRMS (Carlo Erba 1108-Micromass CF-Isochrom) with a measurement error of 0.1 $\delta^{13}C_{V\text{-}PDB}$ units. I compared carbon isotope discrimination between the males and hermaphrodites whose photosynthesis and water potential had been monitored, using a MIXED linear model.

Vegetative characters

I estimated tree size at 196 individuals from the 5 populations (20 males+21 hermaphrodites in Alzira, 11+23 in Bicorp, 14+22 in Bunyol, 22+22 in Font Roja, 21+20 in Sella) and to examine differences in size between genders and populations, used a MIXED linear model.

DBH is not a good estimator of size in this species because individuals resprout multiple stems following disturbances such as fires or coppicing. Therefore, I estimated plant size by measuring tree height and the number of trunks with diameter > 10 cm. The number of trunks was analysed by means of generalized linear models with a Poisson distribution of errors and the canonical link. The dispersion parameter was estimated and included in the model to account for overdispersion. The model was run in the GLM procedure of the R 1.5.1 statistical package (Ihaka and Gentleman 1996).

I measured current-year shoot growth on five branches from the same individuals where physiological measures were taken. Shoot growth was log-transformed to achieve normality and homoscedasticity and analysed by means of a mixed linear model with gender and individual nested within gender as the factors.

Reproductive characters

I estimated the number of pollen grains per anther with a haemocytometer of $0.0025\ \text{mm}^2 \times 0.1$ mm depth (Neubauer Improved, Marienfeld, Germany) in 1–4 flowers of 27 trees randomly selected in the plot (14 males and 13 hermaphrodites) from the Font Roja population. I compared the mean number of pollen grains per flower between genders by means of a GLM with Poisson distribution, canonical link function and inclusion of the dispersion parameter.

I counted the number of flowers per inflorescence in one randomly selected inflorescence from each of the same individuals for which pollen grain quantity was estimated. Data were analysed following the statistical model described for the number of pollen grains.

I counted the number of inflorescences per tree each spring from 1999 to 2002 on the first fourty adult individuals found in a rectangular plot from each of four populations (Font Roja, Bunyol, Bicorp, Alzira). These individuals were tagged in the spring of 1999, with similar numbers of males and hermaphrodites, and included several adult non-flowering trees. After four years without flowering, the gender of some trees remained unknown and therefore they were excluded from the analysis. In addition, the destruction of trees and tags reduced the sample size from 160 to 138 trees (58 males and 80 hermaphrodites). I analysed the number of inflorescences per tree by means of a repeated measures analysis with autoregressive of order 1 covariance structure in which time (year) was the repeated factor, and gender and population the fixed factors.

Standard errors are shown with the mean throughout the text.

Results

Physiological characters

Photosynthetic rate varied during the study period (time $F_{4,59.1} = 12.27$; $p < 0.001$) and was significantly associated with the photosynthetic active radiation arriving at the leaf (PAR $F_{1,80.6} = 20.88$; $p < 0.001$). However, the genders did not differ in mean photosynthesis rate (gender $F_{1,23.5} = 0.36$; $p = 0.55$) or in the monthly rhythm of photosynthesis (gender × time $F_{4,49.5} = 0.47$; $p = 0.76$) (Fig. 2)

Water potential was significantly lower (more negative) in hermaphrodites than males (gender $F_{1,29.11} = 4.89$; $p = 0.03$). Despite the monthly variation in water potential ($F_{4,58.73} = 44.23$; $p < 0.001$), gender differences were maintained over time (gender × time $F_{4,58.73} = 0.29$; $p = 0.88$) (Fig. 3).

These photosynthesis and water potential patterns indicate that hermaphrodites are able to maintain similar photosynthesis rates to those of males, but at lower water potentials (i.e. hermaphrodites are more tolerant to dehydration than males). This intersexual physiological difference is corroborated by the fact that photosynthesis was more reduced by decreasing water potentials in males (slope$\pm$se $= -0.015 \pm 0.007$) than in hermaphrodites (slope$\pm$se $= -0.008 \pm 0.007$, the regression slopes are significantly different; $F_{1,42} = 6.04$; $p = 0.02$; data from all the months were pooled except measures taken at a PAR < 400 that were discarded because photosynthesis may be very limited at low irradiance levels).

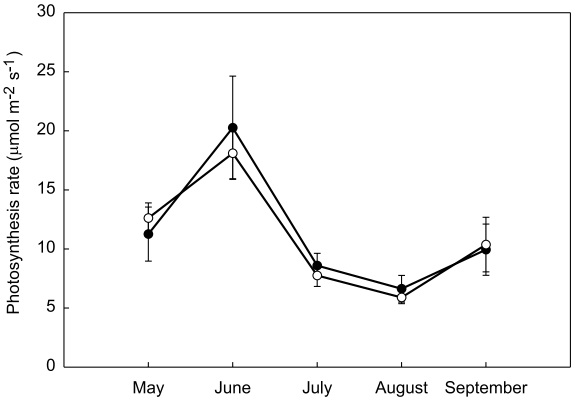

Fig. 2. Change in photosynthetic rate (mean$\pm$SE) of *Fraxinus ornus* males (closed circles) and hermaphrodites (open circles) in the Font Roja population over the 2002 growing season.

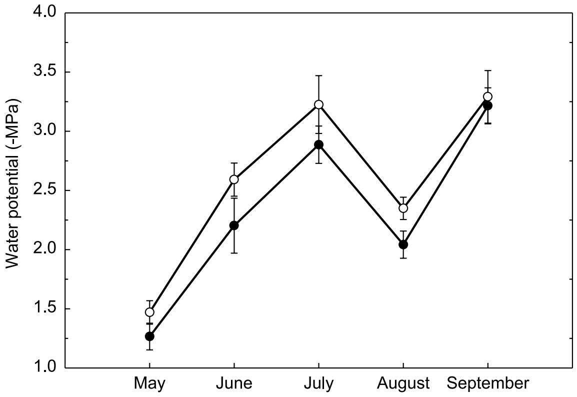

Fig. 3. Change in shoot water potential (mean ± SE) of *Fraxinus ornus* males (closed circles) and hermaphrodites (open circles) in the Font Roja population over the 2002 growing season.

Carbon isotope discrimination was also associated with gender, with hermaphrodites displaying significant lower (more negative) values (− 27.07 ± 0.21) than males (− 26.26 ± 0.24) ($F_{1,17} = 6.11$; $p = 0.02$). This difference means that hermaphrodites had lower integrated water use efficiency than males.

Vegetative characters

The average tree height was similar between males (3.67 ± 0.11 m) and hermaphrodites (3.69 ± 0.11 m) ($F_{1,182} = 0.03$; $p = 0.85$). Although the mean tree height was also similar across populations ($F_{4,182} = 0.64$; $p = 0.63$), there was a significant interaction between gender and population ($F_{4,182} = 3.12$; $p = 0.02$).

The mean number of trunks per tree (4.73 ± 0.33) was similar between genders ($t = 0.51$; df = 192; $p = 0.61$) and populations ($t = 0.58$; df = 192; $p = 0.56$). This pattern was consistent across populations (gender × population $t = 0.01$; df = 192; $p = 0.99$).

There were individual differences in current-year shoot growth ($F_{17,76} = 3.34$; $p < 0.001$), but no difference between genders ($F_{1,76} = 0.82$; $p = 0.37$). Male and hermaphrodite growth values were 2.94 ± 0.25 cm and 4.02 ± 0.79 cm, respectively.

Reproductive characters

Gender was constant in all the trees monitored across the four years in the four study populations.

The mean number of pollen grains per flower was not significantly different between the genders (65 000 ± 4300 for males and 58 000 ± 5500 for hermaphrodites, $t = -1.02$, df = 25; $p = 0.32$). Similarly, no differences in the number of flowers per inflorescences were found between males (890 ± 150) and hermaphrodites (860 ± 600, $t = -0.25$, df = 25; $p = 0.88$).

Males produced a significantly higher number of inflorescences than hermaphrodites (25.2 ± 3.5 vs 15.7 ± 2.9) and this effect (gender) was consistent across years (non-significant interaction year × gender; Fig. 4), populations (non-significant interaction gender × population), and the combination of both (non-significant interaction year × population × gender, Table 1). Masting behaviour is shown by the significant interannual fluctuations in the number of inflorescences (year, Table 1). Populations differed not only in the mean number of inflorescences per tree (population), but also in the interannual rhythm of inflorescence production (year × population).

Discussion

Intersexual differences in some physiological and reproductive traits, but not in vegetative traits were found in the androdioecious species *Fraxinus ornus*. Photosynthesis was similar between both genders, but hermaphrodites had lower water potential than males, suggesting that hermaphrodites consume more water per carbon unit fixed, i.e they have lower water use efficiency. This conclusion is supported by the time-integrative measure of water use efficiency provided by carbon isotope discrimination data. The lower water potential of the hermaphrodites together with the lower water use efficiency inferred from carbon isotope discrimination data, suggests that hermaphrodites are more drought tolerant than males. This might be achieved through an increased capacity to maintain stomata open at lower water potentials (i.e. osmotic adjustment and/or change in cell wall elasticity), as has been reported between

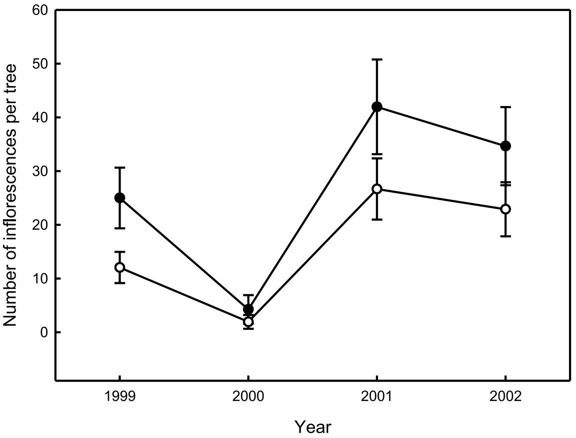

Fig. 4. Change in inflorescence production (mean ± SE) of *Fraxinus ornus* males (closed circles) and hermaphrodites (open circles) in the five study populations.

Table 1. Repeated measures analysis of the number of inflorescences produced over four years by *Fraxinus ornus* males and hermaphrodites in four different populations.

Source	Numerator df	Denominator df	F
Intercept	1	185.5	115.9**
Pop	3	183.8	7.5**
Gender	1	183.5	5.2*
Year	3	386.8	18.9**
Pop × gender	3	183.8	0.2^{ns}
Pop × year	9	387.3	14.8**
Gender × year	3	386.8	1.0^{ns}
Pop × gender × time	9	387.2	0.5^{ns}

ns: non significant; * $p < 0.05$; ** $p < 0.001$.

males and females in some dioecious species (Dawson and Bliss 1989).

Gender specific patterns of carbon isotope discrimination have been found in other dioecious species. In some of these species (*Ilex aquifolium, Phoradendron juniperinum and Acer negundo*), male plants′ carbon isotope discrimination was lower, and therefore water use efficiency higher, than females, especially under stressful conditions (Dawson and Ehleringer 1993, Marshall et al. 1993, Retuerto et al. 2000). In other species, like *Salix arctica,* the reverse situation was true; females having lower carbon discrimination rates than males (Dawson and Bliss 1989, Jones et al., 1999), while in others, no intersexual difference in carbon isotope has been found (*Simmondsia chinensis*; Kohorn et al. 1994). Thus, evidence for greater water use efficiency in females from dioecious species is mixed (Dawson and Geber 1999).

The only evidence at present of gender-differential physiology in non-dioecious, sexually dimorphic species is that of *Wurmbea dioica* (Case and Barrett 2001) where, contrary to expectation, carbon isotope discrimination was higher in cosexuals than in unisexuals (especially males). The only physiological study of an androdioecious species is on the annual herb *Mercurialis annua* where there was no gender differences in carbon assimilation rates (Sleeman et al. 2002). However, the present study of *F. ornus* on gender-differences in physiology on an androdioecious perennial species, reveals that hermaphrodites are more drought tolerant than males. These results suggest that natural selection has favoured different physiological strategies in each of the genders to cope with the different costs of reproduction.

The lack of gender-related differences in size and shoot growth in *F. ornus* suggests that males do not outperform hermaphrodites vegetatively. Indeed, vegetative outperformance seems to be a rare compensation mechanism in gynodioecious species. Seven of 10 gynodioecious species reviewed by Obeso (2002) showed no gender differences in biomass, relative growth rate or plant size. Vegetative traits may be similar between genders if costs of reproduction in hermaphrodites are not higher than those of unisexuals. For example, costs may be similar if the timing of investment in each sexual function differs (Goldman and Willson 1986), e.g. if males invest more in flowering, then hermaphrodites may invest more in photosynthetic tissues early in the season and thereby acquire more resources. Subsequently, fruit abortion and photosynthesis of fruits may help to reduce female costs (Bazzaz et al. 1979, Stephenson 1981). *Fraxinus ornus* abort 37±13% of initiated fruits (averaged from 4 populations during two years), and samaras are green and photosynthetically active when growing (M. Verdú, pers. obs.). Despite these mechanisms to reduce female costs in *F. ornus* hermaphrodites, vegetative outperformance is not produced and therefore, another alternative hypothesis is that *F. ornus* males may be reallocating the resources "saved" by the absence of the female function into greater male reproduction. Higher fecundity rates of males relative to hermaphrodites occurred in *F. ornus*, where males have 1.6 more inflorescences than hermaphrodites. Because the number of flowers per inflorescence and the number of pollen grains per flower did not differ between genders, a higher number of inflorescences suggests a greater reproductive investment at the flowering stage. This is similar to gynodioecious species, where higher fecundity rates of females relative to hermaphrodites have been frequently described (Shykoff 1988, Stevens 1988, Jordano 1993, Maki 1996, Wolfe and Shmida 1997, Shykoff et al. 2003, Alonso and Herrera 2001).

In androdioecious populations, males are expected to make more than twice the genetic contribution through their male function compared to hermaphrodites (Lloyd 1975, Charlesworth 1984). This contribution can be made either through a higher pollen dispersal, or through a higher siring success of dispersed pollen (Pannell 2002a). The first condition is fulfilled in *F. ornus*, where males produce more flowers, as shown here. The second condition is also fulfilled in *F. ornus*, where experimental crosses have shown that males sire more than twice the number of seeds than do hermaphrodites (Dommée et al. 1999, Verdú, unpubl.). These facts implicate functional androdioecy. However, the observed 1:1 sex ratios do not fit well with the theoretical expectation of hermaphrodite-biased sex ratios of the androdioecy models and suggest functional dioecy (Charlesworth 1984). Further work will clearly be needed to resolve this inconsistency. Particularly useful will be estimates of siring success by hermaphrodites in the wild (Vassiliadis et al. 2002).

Despite the intersexual differences shown here, nothing is known about the constraints that gender-specific physiological strategies may impose on the maintenance of androdioecy. Certainly, the fact that males and hermaphrodites in *F. ornus* differ in physiological as well as reproductive investment traits suggests that the former need to be incorporated into accounts of the evolution and maintenance of a male-hermaphrodite polymorphism.

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References

Alonso, C. and Herrera, C. M. 2001. Neither vegetative nor reproductive advantages account for high frequency of male-steriles in southern spanish gynodioecious *Daphne laureola* (Thymelaeaceae). – Am. J. Bot. 88: 1016–1024.

Akimoto, J., Fukuhara, T. and Kikuzawa, K. 1999. Sex ratios and genetic variation in a functionally androdioecious species, *Schizopepon bryoniaefolius* (Cucurbitaceae). – Am. J. Bot. 86: 880–886.

Aronne, G. and Wilcock, C. C. 1992. Breeding system of *Phillyrea latifolia* L. and *Phillyrea angustifolia* L: evidence for androdioecy. – Giornale Botanico Italiano 126: 263.

Bazzaz, F. A., Carlson, R. W. and Harper, J. L. 1979. Contribution to reproductive effort by photosynthesis of flowers and fruits. – Nature 279: 554–555.

Boecklen, W. J., Price, P. W. and Mopper, S. 1990. Sex and drugs and herbivores: sex–biased herbivory in arroyo willow (*Salix lasiolepis*). – Ecology 71: 581–588.

Case, A. L. and Barrett, S. C. H. 2001. Ecological differentiation of combined and separate sexes of *Wurmbea dioica* (colchicaceae) in sympatry. – Ecology 82: 2601–2616.

Charlesworth, D. 1984. Androdioecy and the evolution of dioecy. – Biol. J. Linn. Soc. 22: 333–348.

Correia, O. A. and Diaz Barradas, M. C. 2000. Ecophysiological differences between male and female plants of *Pistacia lentiscus*. – Plant Ecol. 149: 131–142.

Dawson, T. E. and Bliss, L. C. 1989. Patterns of water use and the tissue water relations in the dioecious shrub, *Salix arctica*: the physiological basis for habitat partitioning between the sexes. – Oecologia 79: 332–343.

Dawson., T. E. and Ehleringer, J. R. 1993. Gender-specific physiology, carbon isotope discrimination, and habitat distribution in boxelder, *Acer negundo*. – Ecology 74: 798–815.

Dawson, T. E. and Geber, M. A. 1999. Dimorphism in physiology and morphology. – In: Geber, M. A., Dawson, T. E. and Delph, L. F. (eds), Gender and sexual dimorphism in flowering plants. Springer, pp. 175–215.

Delph, L. F. 1999. Sexual dimorphism in life history. – In: Geber, M. A., Dawson, T. E. and Delph, L. F. (eds), Gender and sexual dimorphism in flowering plants. Springer, pp. 149–173.

Dommée, B., Geslot, A., Thompson, J. D. et al. 1999. Androdioecy in the entomophilous tree *Fraxinus ornus* (Oleaceae). – New Phytol. 143: 419–426.

Goldman, D.A. and Willson, M F. 1986. Sex allocation in functionally hermaphroditic plants: a review and critique. – Bot. Rev. 52: 157–194.

Ihaka, R. and Gentleman, R. 1996. R: a language for data analysis and graphics. – J. Computational Graphical Stat. 5: 299–314.

Ishida, K. and Hiura, T. 1998. Pollen fertility and flowering phenology in an androdioecious tree, *Fraxinus lanuginosa* (Oleaceae), in Hokkaido, Japan. – Int. J. Plant Sci. 159: 941–947.

Jones, M. H., Macdonald, S. E and Henry, G. H. R. 1999. Sex- and habitat-specific responses of a high arctic willow, *Salix arctica*, to experimental climate change. – Oikos 87: 129–138.

Jordano, P. 1993. Pollination biology of *Prunus mahaleb* L.: deferred consequences of gender variation for fecundity and seed size. – Biol. J. Linn. Soc. 50: 65–84.

Kohorn, L. U., Goldstein, G. and Rundel, P. W. 1994. Morphological and isotopic indicators of growth environment: variability in $\delta^{13}C$ in *Simmondsia chinensis*, a dioecious desert shrub. – J. Exp. Bot. 45: 1817–1822.

Laguna, E. and García, M. 1988. El parque natural del carrascal de la Font-Roja. – Vida Silvestre 63: 42–48.

Lange, O. L., Tenhunen, J. D. and Braun, M. 1982. Midday stomatal closure in Mediterranean type selerophylls under simulated habitat conditions in an environmental chamber. I. Comparison of the behaviour of various European Mediterranean species. – Flora 172: 563–579.

Lepart, J. and Dommée, B. 1992. Is *Phillyrea angustifolia* L. (Oleaceae) an androdioecious species? – Bot. J. Linn. Soc. 108: 375–387.

Liston, A., Rieseberg, L. H. and Elias, T. S. 1990. Functional androdioecy in the flowering plant *Datisca glomerata*. – Nature 343: 641–642.

Lloyd, D. G. 1975. The maintenance of gynodioecy and androdioecy in angiosperms. – Genetica 45: 325–339.

Lloyd, D. G. and Webb, C. J. 1977. Secondary sex characters in plants. – Bot. Rev. 43: 177–216.

Maki, M. 1996. Differences in plant size and flower production between hermaphrodites and females of two gynodioecious *Chionographis* (Liliaceae). – Can. J. Bot. 74: 150–153.

Marshall, J. D., Dawson, T. E. and Ehleringer, J. R. 1993. Gender-related differences in gas exchange are not related to habitat preferences in the xylem-tapping mistletoe, *Phoradendron juniperinum* (Viscaceae). – Am. J. Bot. 80: 641–645.

Obeso, J. R. 2002. The costs of reproduction in plants. – New Phytol. 155: 321–348.

Obeso, J. R., Alvarez-Santullano, M. and Retuerto, R. 1998. Sex-ratios, size distributions, and sexual dimorphism in the dioecious tree *Ilex aquifolium* (Aquifoliaceae). – Am. J. Bot. 85: 1602–1608.

Pannell, J. R. 1997. Widespread functional androdioecy in *Mercurialis annua* L. (Euphorbiaceae). – Biol. J. Linn. Soc. 61: 95–116.

Pannell, J. R. 2001. A hypothesis for the evolution of androdioecy: the joint influence of reproductive assurance and local mate competition in a metapopulation. – Evol. Ecol. 14: 195–211.

Pannell, J. R. 2002a. The evolution and maintenance of androdioecy. – Annu. Rev. Ecol. Syst. 33: 397–425.

Pannell, J. R. 2002b. What is functional androdioecy? – Funct. Ecol. 16: 862–865.

Pannell, J. R. and Ojeda, F. 2000. Patterns of flowering and sex-ratio variation in the Mediterranean shrub *Phillyrea angustifolia* (Oleaceae): implications for the maintenance of males with hermaphrodites. – Ecol. Lett. 3: 495–502.

Philbrick, C. T. and Rieseberg, L. H. 1994. Pollen production in the androdioecious *Datisca glomerata* (Datiscaceae): implications for breeding system equilibrium. – Plant Species Biol. 9: 43–46.

Putwain, P. and Harper, J. 1972. Studies in the dynamics of plant populations. V. Mechanisms governing the sex ratio in *Rumex acetosa* and *R. acetosella*. – J. Ecol. 60: 113–129.

Retuerto, R., Fernández Lema, B., Rodríguez Roiloa, S. et al. 2000. Gender, light and water effects in carbon isotope discrimination, and growth rates in the dioecious tree Ilex aquifolium. – Funct. Ecol. 14: 529–537.

Rundel, P. W., Ehleringer, J. R. and Nagy, K. A. 1988. Stable isotopes in ecological research. – Springer-Verlag.

Saavedra, F. and Douglass, L. 2002. Using mixed models in SAS for ecological analyses. – Bull. Ecol. Soc. Am. 83: 180–182.

Sakai, S. 2001. Thrips pollination of androdioecious *Castilla elastica* (Moraceae) in a seasonal tropical forest. – Am. J. Bot. 88: 1527–1534.

Shykoff, J. A. 1988. Maintenance of gynodioecy in *Silene acaulis* (Caryophyllaceae): stage-specific fecundity and viability selection. – Am. J. Bot. 75: 844–850.

Shykoff, J. A., Kolokotronis, S., Collin, C. L. et al. 2003. Effects of male sterility on reproductive traits in gynodioecious plants: a meta-analysis. – Oecologia 135: 1–9.

Sleeman, J. D., Dudley, S. A., Pannell, J. R. et al. 2002. Responses of carbon acquisition traits to irradiance and light quality in *Mercurialis annua* (Euphorbiaceae): evidence for weak integration of plastic responses. – Am. J. Bot. 89: 1388–1400.

Stephenson, A. G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. – Annu. Rev. Ecol. Syst. 12: 253–279.

Stevens, D. P. 1988. On the gynodioecious polymorphism in *Saxifraga granulata* L. (Saxifragaceae). – Biol. J. Linn. Soc. 35: 15–28.

Tapper, P.-G. 1992. Irregular fruiting in *Fraxinus excelsior*. – J. Veg. Sci. 3: 41–46.

Tapper, P.-G. 1996. Long-term patterns of mast fruiting in *Fraxinus excelsior*. – Ecology 77: 2567–2572.

Tenhunen, J. D., Lange, O. L. and Braun, M. 1981. Midday stomatal closure in mediterranean type sclerophylls under simulated habitat conditions in an environmental chamber.II. Effect of the complex of leaf temperature and air humidity on gas exchange of *Arbutus unedo* and *Quercus ilex*. – Oecologia 50: 5–11.

Traveset, A. 1994. Reproductive biology of *Phillyrea angustifolia* L. (Oleaceae) and effect of galling-insects on its reproductive output. – Bot. J. Linn. Soc. 114: 153–166.

Tutin, T. G., Heywood, V. H., Burges, N. A. et al. 1972. Flora Europaea, Vol. 3, Diapensiaceae to Myoporaceae. – Cambridge Univ. Press.

Vassiliadis, C., Lepart, J., Saumitou-Laprade, P. et al. 2000. Self-incompatibility and male fertilization success in *Phillyrea angustifolia* (Oleaceae). – Int. J. Plant Sci. 161: 393–402.

Vassiliadis, C., Saumitou-Laprade, P., Lepart, J. et al. 2002. High male reproductive success of hermaphrodites in the androdioecious *Phillyrea angustifolia*. – Evolution 56: 1362–1373.

Wallander, E. 2001. Evolution of wind-pollination in *Fraxinus* (Oleaceae): an ecophylogenetic approach. PhD thesis. – Göteborg Univ, Göteborg, Sweden.

Wolfe, L. M. and Shmida, A. 1997. The ecology of sex expression in a gynodioecious Israeli desert shrub (*Ochradenus baccatus*). – Ecology 78: 101–110.