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Paternal effects on functional gender account for cryptic dioecy in a perennial plant

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Natural selection operates on the mating strategies of hermaphrodites through their functional gender, i.e. their relative success as male versus female parents. Because functional gender will tend to be strongly influenced by sex allocation, it is often estimated in plants by counting seeds and pollen grains. However, a plant's functional gender must also depend on the fate of the seeds and pollen grains it produces. We provide clear evidence of a paternal effect on the functional gender of a plant that is independent of the resources invested in pollen. In the Mediterranean tree *Fraxinus ornus*, males coexist with hermaphrodites that disperse viable pollen and that sire seeds; the population would thus appear to be functionally androdioecious. However, we found that seedlings sired by hermaphrodites grew significantly less well than those sired by males, suggesting that hermaphrodites may be functionally less male than they seem. The observed 1 : 1 sex ratios in *F. ornus*, which have hitherto been difficult to explain in the light of the seed-siring ability of hermaphrodites, support our interpretation that this species is cryptically dioecious. Our results underscore the importance of considering progeny quality when estimating gender, and caution against inferring androdioecy on the basis of a siring ability of hermaphrodites alone.

Keywords: sex allocation; cryptic dioecy; inbreeding depression; gynodioecy; hermaphroditism

1. INTRODUCTION

The notorious diversity of plant sexual systems (reviewed in Barrett 2002) is reflected in the bewildering array of terms used to describe it. This applies particularly to the categories of gender and gender polymorphisms, where populations comprise mixtures of individuals with different sex-allocation strategies (reviewed in Sakai & Weller 1999). However, as Lloyd (1980) pointed out, these categories are often more apparent than real, and his development of a quantitative and functional framework for the assessment of gender in plants was an important milestone in the maturation of the study of plant sexual systems in general. Lloyd (1980) made a critical distinction between the phenotypic disposition of individuals in terms of their sex allocation, and their functional gender in terms of their realized reproductive success through male versus female function. These two measures may be nearly equivalent in many populations, but they need not be so in principle. The functional gender of a hermaphrodite, for example, will depend on its production and dispersal of pollen grains and their success at fertilizing ovules, but also on the survival to reproductive maturity of the resulting progeny.

The availability of highly polymorphic molecular markers for paternity analysis has revolutionized the potential for functional analysis of gender in plants, and numerous studies now show the importance of phenology, proximity to neighbours, and size in determining male mating success (e.g. Meagher 1991; Nason *et al.* 1998; Elle & Meagher 2000; Morgan & Conner 2001; Vassiliadis *et al.* 2002). However, such studies tend to equate male fitness with siring success by tacitly assuming the absence of paternal effects on progeny growth and viability. In the absence of evidence to the contrary, this is probably a reasonable assumption. However, in the present paper we present data from a long-lived plant species, *Fraxinus ornus*, where this assumption is clearly not met. Apart from emphasizing the importance of progeny viability in determining the functional gender of parents, our results also help to resolve a conflict that has emerged between theory and observation in recent studies of the sexual system of this and related species.

Fraxinus ornus is one of the very few plant and animal species in which males co-occur with hermaphrodites (androdioecy) (Dommée *et al.* 1999; Verdú 2004). The species is particularly interesting because it belongs to a family, the Oleaceae, in which androdioecy has putatively evolved from hermaphroditism several times independently (Wallander 2001; Pannell 2002*a*). Theoretical models indicate that the evolution of androdioecy from hermaphroditism is less easily explained than from dioecy; compare, for example, the conclusions reached by Lloyd (1975), Charlesworth & Charlesworth (1978) and Charlesworth (1984), who considered the evolution of androdioecy from hermaphroditism, with those of Pannell (2001) and Wolf & Takebayashi (2004), who considered the breakdown of dioecy. Indeed, the few well-established cases of androdioecy all appear to have evolved from dioecy (Pannell 2002*a*; Wolf & Takebayashi 2004). *Fraxinus ornus* and its relatives in the Oleaceae thus present putative examples of androdioecy

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that are in particular need of explanation from a functional point of view.

Especially puzzling in these species is the observation of sex ratios that are close to 1 : 1, with males sometimes more frequent than hermaphrodites (Dommée *et al.* 1999; Verdú 2004). This is inconsistent with an interpretation of functional androdioecy, in which males must be the minority gender (see Pannell 2002*b*), and it strongly implicates cryptic dioecy, with hermaphrodites that are, in fact, functionally female (Charlesworth 1984; Mayer & Charlesworth 1991). However, studies of *F. ornus* (Dommée *et al.* 1999, and this paper), and the related *F. lanuginosa* (Ishida & Hiura 1998) and *Phillyrea angustifolia* (Lepart & Dommée 1992; Traveset 1994; Vassiliadis *et al.* 2000), have found that, although pollen produced by hermaphrodites is less able to fertilize ovules than that produced by males, hermaphrodites can, and do, sire seeds. Indeed, in a paternity analysis within a small population of *P. angustifolia*, Vassiliadis *et al.* (2002) recently showed that hermaphrodites actually sired as many seeds as did males under natural pollination conditions.

We argue that the discrepancy between theory and observation in putatively androdioecious populations of the Oleaceae may be due to a failure to consider all critical components of fitness that contribute to the relative siring success of hermaphrodites. For a population of *F. ornus* we confirm earlier observations of reduced male fertility of hermaphrodite pollen. We then present the first analysis, to our knowledge, of the effect of sire on progeny performance in a morphologically androdioecious plant, and we show that seedlings sired by hermaphrodites are inferior to those sired by males. To the extent that this may imply reduced survivorship to reproductive maturity by hermaphrodite-sired progeny, the result immediately suggests that *F. ornus* is cryptically dioecious. This is consistent with observed 1 : 1 sex ratios, and it raises interesting questions about the proximate cause and the broader evolutionary significance of the observed paternal effects on progeny fitness.

2. MATERIAL AND METHODS

(a) *Study site and species*

The study was carried out in the Font Roja Natural Park in eastern Spain, where *F. ornus* occurs as a small tree (maximum height *ca.* 3.5 m) together with *Quercus ilex*, *Acer opalus*, *Quercus faginea* and *Sorbus aria* (for further details on the study area, see Laguna & García 1988). *Fraxinus ornus* flowers in spring and is pollinated by both insects and wind (Wallander 2001). Fruits ripen in autumn, and seeds germinate in the following spring.

(b) *Experimental pollinations*

Experimental pollinations were performed on 10 randomly selected hermaphrodites found within a 5 ha plot in Spring 2002. On each hermaphrodite, 16–20 inflorescences were randomly assigned among the following treatments in a balanced design: (i) pollination with pollen from males; (ii) pollination with pollen from other hermaphrodites; and (iii) self-pollination. Prior to flowering, all the inflorescences were isolated in pollen-proof bags and periodically visited to check for stigma receptivity. When stigmas were receptive, pollen-bearing inflorescences from the relevant pollen donors were shaken within the pollination bags. Because of variation in the level of cross-compatibility among individuals (Dommée *et al.* 1999), we pooled pollen from an inflorescence taken from each of five different fathers in each pollination treatment. To ensure that similar amounts of pollen were used in all treatments, we also used five inflorescences from the same tree for the self-pollination treatment; there are no differences in the number of pollen grains per flower or in the number of flowers per inflorescence between males and hermaphrodites (Verdú 2004), so the amount of pollen used for each of these treatments was also similar. All pollinations were performed over a period of two weeks. Emasculation of hermaphrodite flowers is not possible in *F. ornus* because of the high number and small size of the flowers (Dommée *et al.* 1999). To discriminate between self- and outcrossed fertilizations, we thus determined the paternity of seeds from the cross-pollination treatments using molecular markers (see § 2f).

(c) *Siring success*

We compared the proportions of seeds sired as a result of male-, hermaphrodite- and self-pollinations using a Generalized Linear Mixed Model via Penalized Quasi-Likelihood (glmmPQL), with a Poisson distribution of errors and a canonical link. Additionally, the effect of sire on samara fresh weight (600 samaras sampled, with 20 per treatment and hermaphrodite mother) was tested using the same model on log-transformed data and using a Gaussian distribution of errors. Pollination treatment was analysed as a fixed factor, the hermaphrodite mother as a random clustering factor, and a dispersion parameter was included in the model to account for overdispersion. The model was run in the glmmPQL procedure of the MASS library on the R statistical package (Ihaka & Gentleman 1996; Venables & Ripley 2002). Note that as we were interested in the effects of the sire treatment on the number and quality of progeny produced by a random sample of mothers, we did not consider covariance parameter estimates and associated significance tests, although significant variation between mothers might be expected (for a similar rationale and procedure see Herrera 2000).

(d) *Seed germination*

We compared the germination of seeds (samaras) sired by males, hermaphrodites or through self-fertilization, following the methodology developed by Piotto (1994). One hundred samaras per mother and pollination treatment were stratified in Autumn 2002, for three weeks at 20 °C followed by eight weeks at 4 °C (total number of samaras was 3000). After stratification, in the Spring of 2003, samples of 25 samaras each were placed on moist filter paper in Petri dishes and incubated in the dark under a regime of 25 °C (8 h) and 5 °C (16 h). Germination (radicle emergence from the samara) was scored every 2–3 days. The effect of sire on germination curves was analysed by means of a Cox proportional-hazards regression, with the hermaphrodite mother treated as a clustering factor, using the survival library of the R package (Ihaka & Gentleman 1996). The final germination percentage was compared between the pollination treatments using the model described above for siring proportions, with a binomial distribution of errors.

(e) *Seedling growth*

We transplanted the first 10 germinated samaras per mother and pollination treatment into individual pots (13 cm diameter) with a soil mixture of 40% Vapopeat, 40% coconut coir and 20% expanded perlite (total of 300 seedlings for each treatment combination) and grown up for two months in a glasshouse with daily watering. We then transplanted them into a nursery for the rest of the first growing season and measured their heights once a month. In Autumn 2003 we harvested all seedlings, dried them at 60 °C, and measured their root and shoot dry weights. We used a

Table 1. Means and standard errors for traits of male-sired, hermaphrodite-sired and self-sired progeny of *Fraxinus ornus* from experimental crossings.
(Means shown with different superscripts (*,**,***) are significantly different from one another at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.)

trait	male sire	hermaphrodite sire	self-sire	test	p-value
samara number per inflorescence	$173 \pm 23^{*}$	$82 \pm 18^{**}$	$62 \pm 12^{**}$	$F_{2,91} = 9.54$	0.0002^{a}
samara fresh weight (mg)	$33.2 \pm 1.2^{*}$	$33.9 \pm 0.8^{**}$	$35.6 \pm 0.9^{**}$	$F_{2,586} = 5.83$	0.003^{b}
germination (%)	70.8 ± 2.5	68.0 ± 3.1	64.70 ± 2.8	$F_{2,99} = 2.26$	0.11^{c}
seedling height^{d}	$572.4 \pm 32.8^{*}$	$511.9 \pm 35.2^{**}$	$424.1 \pm 31.4^{***}$		
shoot biomass	$10.9 \pm 1.1^{*}$	$10.1 \pm 1.1^{**}$	$7.9 \pm 0.9^{**}$	$F_{2,225} = 5.17$	0.006^{b}
root biomass	2.9 ± 0.2	2.7 ± 0.2	2.4 ± 0.2	$F_{2,225} = 2.76$	0.06^{b}
total biomass	$13.7 \pm 1.4^{*}$	$12.8 \pm 1.3^{**}$	$10.2 \pm 1.2^{*}$	$F_{2,225} = 4.67$	0.01^{b}

[a] Generalized Linear Mixed Model via Penalized Quasi-Likelihood with a Poisson distribution of errors.

[b] Generalized Linear Mixed Model via Penalized Quasi-Likelihood with a Gaussian distribution of errors.

[c] Generalized Linear Mixed Model via Penalized Quasi-Likelihood with a binomial distribution of errors.

[d] Only final means and s.e. are shown in the table. The longitudinal analysis of seedling height over the study period (figure 2) was performed using a Generalized Estimation Equation procedure; all the three contrasts were statistically significant (male versus hermaphrodite, $p = 0.004$; male versus selfing, $p = 0.000\,001$; hermaphrodite versus selfing, $p = 0.000\,01$).

Generalized Equation Estimation to compare the logarithm of seedling growth in height by means of a repeated measures analysis with an autoregressive covariance structure of order 1 and a Gaussian error distribution. Pollination treatment was analysed as a fixed factor, maternal parent as a random clustering variable, and time (month) as a repeated effects variable. The model was run in the geese procedure of the geepack library on the R package (Ihaka & Gentleman 1996). The effect of treatment on the final dry weight was compared using the model described above for seed weights. Throughout, data are presented as means with one standard error.

(f) *Identification of selfed progeny*

Because we were unable to emasculate inflorescences, we used three nuclear microsatellite loci (see electronic Appendix A for primer and allele information) in a paternity analysis to determine the extent to which seeds from the cross-pollination treatments were in fact self-fertilized. Leaflets were sampled from 164 surviving seedlings and their 10 known mother trees, of which the genotypes for 134 could ultimately be determined. Seedlings were dried in silica gel and stored at −20 °C prior to DNA extraction. Genomic DNA was isolated using a cetyltrimethylammonium bromide (CTAB) procedure, modified from the procedure of Doyle & Doyle (1987), using between 15 and 30 mg of leaf material and eluting in a final volume of 100 μl of TE buffer. DNA extracts were diluted 1 : 10 for PCR, which was performed following the procedure described in Heuertz *et al.* (2001) (see electronic Appendix A). Fluorescent labelling of the forward primers allowed detection of amplification products on an automated DNA sequencer (ABI PRISM 310 DNA sequencer). Sizing of fragments was performed with the software programs GENESCAN 2.1 and GENOTYPER 1.1 (Applied Biosystems) by comparison with an internal size standard (Genescan-400HD Rox). We compared the number of selfed and crossed progeny from male and hermaphrodite pollination treatments using a glmmPQL with a binomial distribution of errors, the canonical link and including the dispersion parameter.

3. RESULTS

(a) *Samara number and weight*

Despite substantial variation between mothers (data not shown), samara production per inflorescence differed significantly among pollination treatments ($F_{2,91} = 9.54$; $p = 0.0002$), with males siring significantly more samaras than hermaphrodites and selfing yielding fewer samaras than hermaphrodites, though this latter difference was not statistically significant (table 1). Samara fresh weight also differed significantly between treatments ($F_{2,586} = 5.83$; $p = 0.003$), with male-sired samaras significantly smaller than those sired by hermaphrodites or produced by selfing, which did not differ from one another (table 1).

(b) *Seed germination*

The mean percentage seed germination across all experimental treatments was 67.6 ± 1.7. The cumulative percentage of seeds germinated was slightly higher for male- than for hermaphrodite-sired seed, which in turn were higher than for self-sired seed, although these differences fell short of statistical significance (table 1). The same order of superiority was displayed by germination rates (figure 1).

(c) *Seedling growth*

Seedlings from male-sired samaras grew significantly taller than those sired by hermaphrodites, which in turn were taller than self-sired seedlings (table 1; figure 2). Shoot and total seedling biomass also differed significantly among sire classes (shoot biomass: $F_{2,225} = 5.17$; $p = 0.0063$; total seedling biomass: $F_{2,225} = 4.67$; $p = 0.01$; table 1); in both cases, the biomass components of male-sired seed were greater than those of hermaphrodite- and self-sired seed (which did not differ from one another). The pattern was similar for root biomass, although the differences fell just short of statistical significance ($F_{2,225} = 2.76$; $p = 0.06$; table 1).

(d) *Progeny paternity*

Eighty-seven per cent of the seedlings from cross-pollinations possessed a microsatellite allele at one or more of the three loci that was not present in their maternal parent,

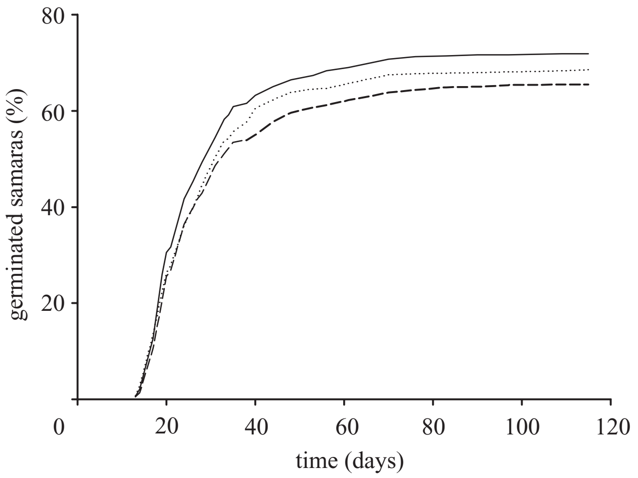

Figure 1. Germination curves for samaras produced under the three different pollination treatments: pollination by male pollen (solid line) , hermaphrodite pollen (dotted line) and self-pollen (dashed line). The curves are not significantly different: likelihood ratio of the Cox regression = 2.32, d.f = 2; $p = 0.31$; $n = 1881$.

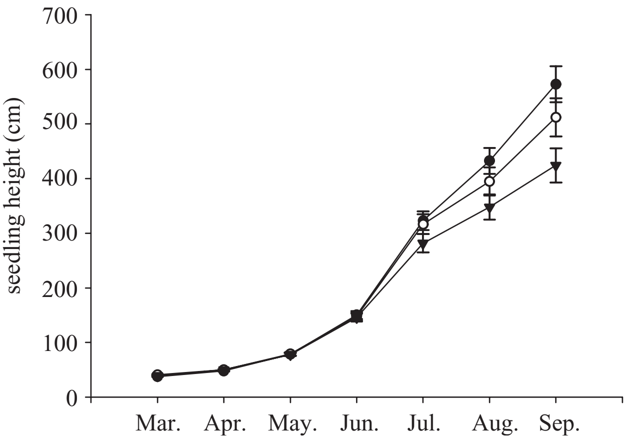

Figure 2. Growth curves of seedlings produced by different pollination treatments: pollination by male pollen (filled circles), hermaphrodite pollen (open circles) and self-pollen (filled inverted triangles). See table 1 for statistical tests.

indicating that they were not produced by selfing. Selfed progeny were equally frequent in the male and hermaphrodite pollination treatments ($F_{1,123} = 0.0007$; $p = 0.98$).

3. DISCUSSION

In this study, we used controlled pollinations and paternity analysis to examine various components of siring success in a morphologically androdioecious population of the tree *F. ornus*. Overall, our results show that males are better sires than hermaphrodites, not only in terms of the average number of seeds they sired in our controlled pollinations, but also in the quality of the offspring produced. We also found that seedlings resulting from self-fertilizations grew less well than outcrossed seeds, whether these were sired by males or hermaphrodites. Together with the 1 : 1 sex ratios observed in natural populations, these results strongly suggest that *F. ornus* is functionally dioecious rather than androdioecious. They also have general implications for the analysis of functional gender, for the interpretation of breeding systems in which males co-occur with hermaphrodites, and for the maintenance of outcrossing in long-lived species. We discuss these three issues in turn below.

(a) *Paternal effects on functional gender*

A plant's functional gender is the direct outcome of its relative genetic contribution made to future generations through its male versus female gametes (Lloyd 1980), and this will be strongly influenced by its sex allocation. Although functional gender will also depend on details of the mating system and pollination biology of the population, the distribution of functional gender alone is both necessary and sufficient to discriminate between sexual systems such as gynodioecy, androdioecy and dioecy. It would be convenient to infer the functional maleness of an individual by determining its paternal contribution to the seeds produced in a given generation, as is commonly done in paternity analyses. However, our finding of paternal effects on the early stages of seedling growth, and by extrapolation on overall plant fitness, indicates that this will not always be sufficient. Rather, functional maleness may be significantly reduced as a result of the differential mortality or inferiority of the progeny it sires. This possibility is implicit in the framework of functional gender analysis, but to our knowledge such paternal effects have never been invoked or explicitly included in models of sex allocation or the evolution of gender dimorphism.

It is known that the maternal contribution, and thus the functional femaleness, of hermaphrodites can be reduced in gynodioecious populations if they produce poorer-quality offspring than do females. However, in both evolutionary models (e.g. Lloyd 1974, 1975; Charlesworth & Charlesworth 1978) and empirical studies of gynodioecy (e.g. Kohn 1988; Delph & Lloyd 1996), the reduced average fitness of progeny produced by hermaphrodites is generally attributed to inbreeding depression following self-fertilization of a proportion of their ovules, whereas the seeds produced by females are always outcrossed. By contrast, although our results indicate that self-fertilization is possible in *F. ornus* (see also Dommée *et al.* 1999), and the level of inbreeding depression appears to be high (see § 4c), we found that the paternal effects on progeny quality were independent of inbreeding.

Maternal effects on offspring success that are independent of inbreeding depression have been widely documented (reviewed in Roach & Wulff 1987; Mousseau & Fox 1998; Delph *et al.* 1999). These are commonly understood in terms of differences between maternal parents in their capacity to provision their offspring, or in terms of cytoplasmically inherited differences (Delph & Mutikainen 2003). There is no indication from our results that the seeds of male sires of *F. ornus* are better provisioned than those sired by hermaphrodites. In fact, we found that male-sired seeds were significantly smaller. Several examples are known from gynodioecious species in which progeny fitness depends on the gender of the maternal parent irrespective of seed size (reviewed in Delph 2004). For example, seeds produced by hermaphrodites show superior growth and survivorship than those produced by females in gynodioecious *Silene acaulis*, even though females produce smaller seeds. Delph (2004) conjectured that this difference in *S. acaulis* may be due to the costs of male-fertility restoration in hermaphrodites. However, similar causes of the paternal effects observed in *F. ornus* seem unlikely, given that paternally inherited genetic elements should be transmitted by both males and hermaphrodites. Unfortunately, in the absence of further data or alternative models, it remains difficult to speculate on causes for the patterns we observe. One possibility is that the low observed pollen fertility of hermaphrodites and the poorer growth of hermaphrodite-sired progeny might be pleiotropic effects of sex-linked genes.

(b) *The effect of functional gender of the sexual system*

Our results are particularly significant for the interpretation of the sexual system of *F. ornus*, and by extension for that of related species that display similar patterns of phenotypic gender (Wallander 2001). Theoretical models of androdioecy express the frequency at which males can be maintained with functional hermaphrodites in terms of the selfing rate of hermaphrodites, the levels of inbreeding depression suffered by selfed progeny, the relative survivorship of males versus hermaphrodites, and the relative siring success of males versus hermaphrodites (Lloyd 1975; Charlesworth & Charlesworth 1978; Charlesworth 1984). This last term must be greater than two for males to persist in the population, and its inverse must approach zero for males to approach a frequency of 0.5, i.e. hermaphrodites must function increasingly only as females. In studies of androdioecy in *Datisca glomerata* (Liston *et al.* 1990; Philbrick & Rieseberg 1994), *Mercurialis annua* (Pannell 1997*a*,*b*), and *Schizopepon bryoniaefolius* (Akimoto *et al.* 1999), estimates of relative siring success have hitherto been based on measures of the relative amount of pollen dispersed by males and hermaphrodites. This implicitly assumes that pollen grains produced by males and hermaphrodites are equally likely to fertilize an ovule, and it is clear from our results that this critical assumption is not met in *F. ornus*.

To illustrate the implications of including paternal effects in a model for the maintenance of androdioecy, let r be the pollen production of males relative to hermaphrodites, v be the ability of hermaphrodite-produced pollen to sire seeds in open competition with pollen produced by males, s be the proportion of seed that is self-fertilized by hermaphrodites, $1-d$ be the probability of surviving to maturity of selfed relative to hermaphrodite-sired outcrossed progeny, and x be the survival of hermaphrodite-sired outcrossed progeny to reproductive maturity relative to that sired by males. Then, by equating expressions for male and hermaphrodite fitness, we can write down an expression for m, the frequency of males in a population at equilibrium:

$$m = \frac{r(1-s) + vx(2ds - s - 2)}{2r\{1 - s + sx(1-d)\} + vx(2ds - s - 1)}. \qquad (4.1)$$

With $v = 1$ and $x = 1$, this expression reduces to equivalent expressions derived by Lloyd (1975) and Charlesworth (1984), who considered the effects of selfing and inbreeding depression but ignored possible differences between males and hermaphrodites in the viability of their pollen or the viability of the progeny that they sire. It is evident from equation (4.1) that as x approaches zero, so m approaches 0.5 independently of s, and that the population thus becomes functionally dioecious. The selfing rate in natural populations of *F. ornus* is not yet known. However, given the low proportion of seed that was self-fertilized in the outcross pollination treatments of this study and the probable high level of inbreeding depression suffered by selfed progeny (see § 4c), the effective selfing rate in natural populations may be negligible, and we might thus set $s = 0$. We calculated $r = 2.04$ as the mean number of pollen grains produced by males relative to that produced by hermaphrodites (1.812; data from Verdú 2004), multiplied by the relative flowering frequency of males versus hermaphrodites (1.12; based on observations of 138 trees over 4 years; M. Verdú, unpublished data). The expected male frequency for a population with these values of s and r, and assuming $v = 1$, is plotted against x in figure 3.

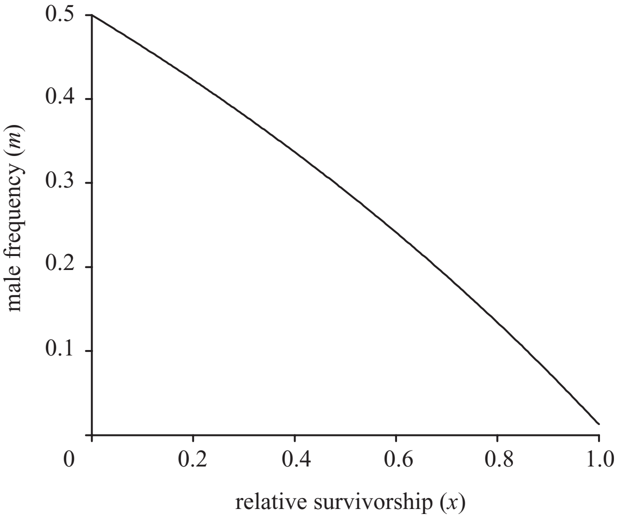

Figure 3. A plot of expression (1), with $s = 0$, $v = 1$, $r = 2.04$, and m, the expected frequency of males in a population, plotted against x, the relative survival of male-sired versus hermaphrodite-sired progeny to reproductive maturity (see text for details).

It is clear from figure 3 that x must be very small to account for the high male frequencies observed in natural populations of this species. At face value, our results fall well short of this requirement, with $x = 0.89$ calculated as the product of the proportion of seeds germinated and the biomass of seedlings after the first season of growth (table 1). However, it is well known that small differentials in growth at the seedling stage may reduce total fitness substantially when compounded over the lifetime of a perennial plant, especially when subjected to asymmetric competition (Weiner 1985; Schmitt & Ehrhardt 1990; Husband & Schemske 1996). It is thus conceivable that the reproductive value of hermaphrodite-sired progeny may become negligible compared with male-sired progeny. Although speculative, this possibility is consistent with the sex ratios actually observed in populations of *F. ornus* and other morphologically androdioecious species in the family. Thus, whereas a paternity analysis for a natural population of the related species *Phillyrea angustifolia* showed that hermaphrodites may sire substantial numbers of seeds, our results from *F. ornus* urge caution against interpreting such data as sufficient evidence for functional androdioecy, particularly as sex ratios in this species often approach equality (although see Pannell & Ojeda 2000). High male frequencies that are difficult to explain in terms of functional androdioecy have also been observed in other species of the genus *Fraxinus* (Ishida & Hiura 1998, 2002; Morand-Prieur *et al.* 2003), and similar caution should be taken in interpreting their sexual systems.

The reduced male function of hermaphrodites in *F. ornus* in some ways mirrors the reduced female function of hermaphrodites in some gynodioecious species (e.g. Lloyd 1976). In both situations, hermaphrodites contribute genes to progeny almost exclusively through one sexual function, so that populations are functionally dioecious or subdioecious and display sex ratios close to 1 : 1. In gynodioecious species, the reduced female function of hermaphrodites is often due to a reduction in seed production and a possible re-allocation of resources to male components of reproduction (Lloyd 1976; Delph & Lloyd 1991; Barrett *et al.* 1999). By contrast, the sexual specialization of hermaphrodites in *F. ornus* towards female function does not appear to be mediated to the same extent by reduced allocation to male function. Although pollen production by hermaphrodites is lower than that by males, the primary cause of their reduced male function is the poorer quality of their pollen and of the progeny they sire. Given that *F. ornus* does not produce nectar, it is possible that functional females produce pollen as a reward for pollinators and that the transition from insect pollination to wind pollination has not yet run its full course (Wallander 2001).

(c) *Inbreeding depression and the maintenance of outcrossing*

Finally, we note that the effect of sire on progeny vigour in the context of cryptic dioecy places us in an unusual position to infer the level of inbreeding depression in *F. ornus*. It is well understood that, for outcrossing to be maintained in a population by selection, it is sufficient for the level of inbreeding depression upon self-fertilization to be greater than *ca.* 0.5 (e.g. Lande & Schemske 1985; Schultz 1999; Holsinger 2000). Our estimate of inbreeding depression in *F. ornus*, based on the product of the proportion of seeds germinated and the biomass of seedlings after the first season of growth, is 0.32. Although lower than the 0.5 threshold this value represents relative growth under the benign conditions of a glasshouse and nursery where the effects of inbreeding depression may be ameliorated (Dudash 1990). Moreover, the poor performance of inbred progeny over the first season is likely to be magnified over subsequent years, as argued above (see also Lande *et al.* 1994; Morgan *et al.* 1997).

The estimation of the lifetime reduction in fitness through inbreeding will typically require detailed measurements of inbreeding depression over the life cycle of a plant (Husband & Schemske 1996; Morgan *et al.* 1997). However, together with the 1 : 1 sex ratios observed in natural populations, our results suggest that hermaphrodite-sired progeny are unlikely to contribute to subsequent generations. In our experiment, selfed progeny performed less well than those sired by outcrossing hermaphrodites, suggesting that they will also fail to reproduce. Lifetime inbreeding depression in *F. ornus* may thus be as high as 100%, more than sufficient for the evolutionary maintenance of dioecy as an outcrossing mechanism.

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