Annals of Botany **110**: 1471–1478, 2012
doi:10.1093/aob/mcs182, available online at www.aob.oxfordjournals.org

ANNALS OF BOTANY Founded 1887

PART OF A SPECIAL ISSUE ON POPULATION BIOLOGY

Do plants adjust their sex allocation and secondary sexual morphology in response to their neighbours?

Julia Sánchez Vilas* and John R. Pannell

Department of Ecology and Evolution, Le Biophore, UNIL-SORGE, University of Lausanne, CH-1015 Lausanne, Switzerland
** For correspondence. E-mail julia.sanchezvilas@gmail.com*

Received: 3 April 2012 Returned for revision: 15 May 2012 Accepted: 21 June 2012 Published electronically: 16 August 2012

• *Background and aims* Changes in the sex allocation (i.e. in pollen versus seed production) of hermaphroditic plants often occur in response to the environment. In some homosporous ferns, gametophytes choose their gender in response to chemical cues sent by neighbours, such that spores develop as male gametophytes if they perceive a female or hermaphrodite nearby. Here it is considered whether a similar process might occur in the androdioecious angiosperm species *Mercurialis annua*, in which males co-occur with hermaphrodites; previous work on a Spanish population of *M. annua* found that individuals were more likely to develop as males at high density.
• *Methods* Using a novel approach to treat plants with leachate from pots containing males or hermaphrodites of *M. annua*, the hypothesis that individuals assess their mating opportunities, and adjust their sex expression accordingly, was tested through an exchange of chemical cues through the soil.
• *Key Results* For the population under study, from Morocco, no evidence was found for soil-signal-dependent sex expression: neither sex ratios nor sex allocation differed among experimental treatments.
• *Conclusions* The results imply either that the Moroccan population under study behaves differently from that previously studied in Spain (pointing to potential geographical variation in plasticity for sex expression), or that our method failed to capture the signals used by *M. annua* for adjustment of sex expression.

Key words: Androdioecy, environmental sex determination, environmental cues, hermaphroditism, phenotypic plasticity.

INTRODUCTION

Because plants are sessile, their success depends critically on an ability to respond plastically to environmental stimuli such as light quality, day length, nutrient availability and herbivory. Such responses have presumably been selected to maximize growth and survivourship in a given context, but to what extent is phenotypic plasticity used to maximize mating and reproductive success? In many animal species, individuals are known to modify their morphology and behaviour in response to local mating opportunities. In some bird species, for example, males change their plumage during the reproductive season (Darwin, 1871; Andersson, 1983). More dramatically, many fish species switch gender in response to the local 'operational sex ratio', with a tendency for females to become male when males are scarce, and vice versa (Krebs, 1976; Andersson, 1994). Given that the mating success of plants, too, should depend on local mate availability, we might expect natural selection to have favoured a similar ability to switch gender in response to perceived opportunities.

Sex choice in response to mating opportunities is now well established for some homosporous fern species, in which all spores are identical in size and develop as male or female gametophytes in response to environmental cues given by neighbours. In particular, spores germinate by default as hermaphrodites or females and immediately begin releasing a hormone, antheridiogen, into their environment. Spores germinating in the presence of antheridiogen, however, sense the proximity of hermaphrodites or females and develop as males (Banks, 1997). The advantages of this strategy of inter-plant communication should, on theoretical grounds (Haig and Westoby, 1988), benefit both genders, and it is therefore perhaps not surprising that a mechanism has evolved to allow it in ferns. Angiosperms are known to communicate with one another via chemical signals. For example, by means of air-borne 'semiochemicals', plants are able to perceive damage caused to other plants in their vicinity due to herbivore attacks, and thus to produce defence substances prior to being attacked themselves (Shulaev *et al.*, 1997; Karban *et al.*, 2000; Baldwin *et al.*, 2006; Heil and Kost, 2006). However, the question remains as to whether angiosperms have evolved ways of using semiochemicals to optimize their sex expression and mating opportunities.

A potential candidate for such a situation is the European wind-pollinated androdioecious herb *Mercurialis annua* (Euphorbiaceae). Although diploid populations of *M. annua* are dioecious, hexaploid populations in the Iberian Peninsula and Morocco are often androdioecious, with males co-occurring with hermaphrodites. Mating in these populations is both frequency- and density-dependent. At low densities, hermaphrodites tend to self-fertilize their progeny and males thus have low siring success, but outcrossing rates are high in dense populations, and males enjoy high siring success (Eppley and Pannell, 2007). Because density fluctuates widely from generation to generation in this annual colonizer of disturbed habitats, one would expect genotypes with an ability to choose their gender on the basis of their population density to have a distinct advantage: individuals should choose to be male in dense mainly-hermaphrodite stands but hermaphrodite in sparse stands, just as is observed in the homosporous ferns referred to above. Indeed, in a study of sex expression in *M. annua*, Pannell (1997*a*) found that male frequency increased with density within an androdioecious population in southern Spain and showed in a further experimental study (Pannell, 1997*b*) that at least some of this variation was due to plasticity in gender expression, with males becoming hermaphrodites at low density. Other work on the species (Dorken and Pannell, 2008) has documented patterns of density-dependent gender expression among hermaphrodites.

Although density appears to have an important influence on the sex expression and sex allocation of individuals of androdioecious *M. annua*, it is not known how individuals perceive density. One possibility is that plants judge their mating prospects in terms of the proximity of neighbours through perception of the red/far red ratio of light attenuated by their leaves. However, such a signal would probably be misleading, because *M. annua* often competes with other species whose presence would only interfere with mating prospects, not enhance them. An alternative possibility is that individuals perceive the presence of conspecific neighbours by communicating with one another, either using volatile compounds released into the air or through signals released into the rhizosphere. Given the precedent for this latter scenario in homosporous ferns (Banks, 1997) and motivated by the density-dependent gender expression reported in *M. annua* (Pannell, 1997*a*, *b*; Dorken and Pannell, 2008), here we test the hypothesis that signals released into the soil by males and hermaphrodites of *M. annua* are able to influence the sex expression of other conspecifics. In the absence of neighbours, it would be advantageous for an individual to express both sexual functions, in order to be able to self-fertilize. In the presence of hermaphrodites, individuals developing as males would have an advantage because of their strong siring ability. In the presence of males, by contrast, individuals should develop as hermaphrodites with an enhanced female function.

We examined the potential effects of soil-borne chemicals produced by males and hermaphrodites of *M. annua* by treating seedlings with leachate from (i.e. water passed through) pots of soil supporting either *M. annua* males or *M. annua* hermaphrodites. In addition, we also treated seedlings with leachate from pots containing only soil, in order to examine the potential effects of density (presence vs. absence of plants) more generally. Specifically, we asked whether there is an effect of leachate source on: (1) the sex ratio (i.e. sex expression of the individuals), (2) the patterns of biomass allocation to reproduction of the individuals and (3) the growth and morphological traits in males and hermaphrodites of *M. annua*. We were prompted to address the second question because hermaphrodites of *M. annua* (Pannell, 1997*a*, *b*; Dorken and Pannell, 2008), and those in many other species (reviewed by Delph and Wolf, 2005) are known to adjust their patterns of sex allocation in response to environmental quality; the possibility that they might do so in response to signals transmitted by neighbours has, to our knowledge, hitherto not been investigated. We addressed the third question because androdioecious *M. annua* is sexually dimorphic, with males being taller than hermaphrodites and dispersing their pollen from erect inflorescence stalks (peduncles) that differ from the subsessile axillary inflorescences of hermaphrodites (Hesse and Pannell, 2011*a*); it is thus possible that the degree of sexual dimorphism might be responsive to signals from neighbours, for example with hermaphrodites expressing more male-like morphology under conditions favouring male fitness. Note that flowering in *M. annua* commences a few weeks after seeds germinate and continues indeterminately during plant growth, with new inflorescences produced in each new leaf axil (Pannell, 1997*c*). There is thus ample opportunity for sex-allocation adjustment as plants acquire information about their environment during their continued growth. Pannell (1997*b*) found that plants often began as males and shifted their allocation to female function later in their lives.

MATERIALS AND METHODS

Experimental design

The population studied by Pannell (1997*a*, *b*) has become locally extinct. We thus used seeds for our experiment collected (in 2004) from androdioecious populations of *Mercurialis annua* between Fez and Rabat in Morocco, in which hermaphrodites are known to be highly plastic in their sex allocation (Dorken and Pannell, 2008, and unpubl. data). We first sowed seeds in 9-cm-diameter pots in a glasshouse at the Department of Plant Sciences, University of Oxford. Germination took place within 6 d. Pots were distributed spatially among 15 blocks on the glasshouse benches, each of which corresponded to the leachate blocks (see below). Within each block, plants were randomly assigned to one of three treatments: male-leachate, hermaphrodite-leachate and control-leachate (60 plants per block, 20 plants per treatment in each block; see experimental set-up in Fig. 1). Plants were watered with experimental treatments when the first pair of true leaves was present (1 week after germination). Plants were watered twice a week with leachate (about 50 mL per plant) for approximately 6 weeks until they were harvested. Additional watering was applied between each application of leachate treatment after the third week of growth. The

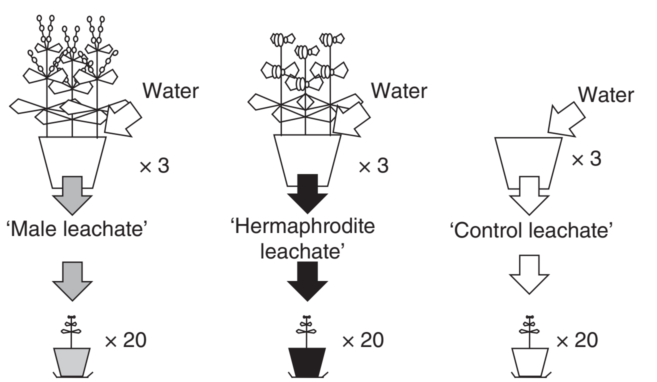

FIG. 1. Schematic diagram representing one of the 15 blocks existing in the experiment. Male, hermaphrodite and control leachate were obtained from collecting water that passed through three pots containing three males, three hermaphrodites and no plants, respectively. Each leachate treatment was randomly applied to a total of 20 seedlings per block.

position of pots within its block was randomly rearranged weekly. Saucers were placed under each pot to avoid leachate contamination among treatments within the block.

Procurement of leachate treatments

Leachate was obtained from 7·5-L pots containing three individuals of *M. annua* of the same sex. These 'leachate donors' were obtained from seedlings from the same source population. They were reared individually over 4 weeks until their gender could be determined, and they were then transplanted into the 7·5-L pots. The leachate donor pots were distributed randomly among the 15 experimental blocks, each block comprising three replicate pots for each of three leachate treatments: leachate from male donors ('male leachate'), from hermaphrodite donors ('hermaphrodite leachate') and from pots containing the same soil but no plants ('control leachate') (see Fig. 1). Leachate was obtained from all pots by watering them until their soil had reached field capacity, and then by continuing to water until we had collected 1 L of leachate flowing from holes in the pots' base. Leachate donors were 5 weeks old when leachate was first collected and applied to the experimental pots. The same set of leachate donors was used for the entire duration of the experiment.

Variables measured

Plants were harvested at 7 weeks after germination, and, for each plant, we measured its height, length of the first pair of branches, length of the three first internodes, petiole and blade length and blade width of the first pair of leaves, above-ground plant dry mass, and dry mass allocated to peduncles and to male and female functions. Mean branch length, mean internode length, and mean petiole, mean blade length and mean blade width of the first pair of leaves were calculated by averaging the corresponding variables. Total, male and female reproductive efforts (i.e. TRE, MRE and FRE) were calculated by dividing the dry mass allocated to the corresponding reproductive function (male flowers and/or female flowers and fruits) by above-ground dry mass. Dry mass of vegetative and reproductive structures was measured after drying the samples to constant mass at 60 °C. As flowering in *M. annua* is indeterminate (producing new flowers in each new leaf axil), allocation to reproduction must be assessed in terms of a plant's female and/or male allocation at a snapshot in time. When we decided to harvest the plants at 7 weeks old, plants were still growing (and already dispersing some seeds and pollen, but also producing new male and female flowers) and have not became pot bound. Previous work has shown that this provides a robust estimate of a plant's total allocation (Pannell, 1997*c*).

For each treatment within each block, we counted the number of males and hermaphrodites. Individuals were classified as males if they produced only pollen and no seeds. Following Pannell (1997*a*), we calculated the pollen production of males relative to that of hermaphrodites (r) for each treatment within each block, as the proportion of above-ground dry mass allocated to pollen by males (π_m) divided by the pollen allocation of hermaphrodites (π_h; i.e. $r = \pi_m/\pi_h$). We also calculated the standardized phenotypic gender for each individual following Lloyd (1980) and Lloyd and Bawa (1984): $G_i = d_i/(d_i + l_iE)$, where d_i is the maternal allocation of individual i and l_i is the paternal allocation of individual i, and $E = \Sigma d_i/\Sigma l_i$ is the ratio of maternal to paternal allocation in the population. Maternal and paternal allocation was measured as dry mass of female and male reproductive structures, respectively.

Data analysis

Sex expression. To analyse the effect of treatment leachate on the proportion of males, we fitted a generalized linear mixed model (GLMM) with binomial errors and logit link function. Analyses were conducted in the R platform (R Development Core Team, 2009), using the glmer function in the lme4 package (Bates *et al.*, 2008). Sex ratio (i.e. male/hermaphrodite ratio), representing the proportion of males per treatment within each block, was set as the response variable, with sample size as the denominator (using the cbind command; see Wilson and Hardy, 2002). Block was included in the analysis as random factor. Differences in the distribution of phenotypic gender between leachate treatments were tested using Kolmogorov–Smirnov two-sample tests.

Growth and allocation measures. Linear mixed-effects models (using the 'lme' function in R) were used to test for differences between the sexes in their response to leachate treatments for growth, morphological and allocation measures (see above for details). Because of correlations among morphological traits (mean branch length, mean internode length, mean petiole length, mean blade length and mean blade width), we performed a principal component analysis (PCA) using the 'prcomp' function in R. The PCA identified two principal components (PC1 and PC2) that explained 67 % of the variation in the morphological data (Table 1), and that were analysed using the linear mixed-effect models. PC1 is mostly related to leaf traits (petiole and blade lengths and blade width), whilst PC2 is highly inversely related to branch and internode length (Table 1). Male reproductive effort, female reproductive effort and biomass allocated to peduncles showed a bimodal distribution, reflecting the inclusion of two sex classes (males and hermaphrodites). In order to use linear mixed models to test for differences on MRE, FRE and allocation to peduncles due to treatments, we thus split the dataset into these two sex categories. To meet the assumptions of normality and homogeneity of variance of linear

TABLE 1. *PCA on five morphological traits of males and hermaphrodites of* M. annua*; the loadings of variables and the proportion of variance explained are reported for the first two principal components*

	PC1	PC2
Mean branch length	0·160	–0·805
Mean internode length	0·401	–0·459
Mean petiole length	0·475	0·098
Mean blade length	0·565	0·186
Mean blade width	0·519	0·311
Cumulative percentage	45	67

models, MRE was square-root transformed and hermaphrodite allocation to male function (r) was $\log_{10}$-transformed. In all linear mixed-effects models, leachate treatment and sex were treated as fixed factors, and block was included in the analysis as random factor. Significance of fixed effects in lme models was examined through F-tests using the procedure 'anova' in R. All statistical tests are presented without adjustment for multiple tests (see Moran, 2003; Nakagawa, 2004).

Standardized major axis (SMA) regression was used to estimate the significance of the relationships between above-ground dry mass and total and male reproductive allocation for each leachate treatment. Correlation coefficients and SMA slopes were calculated using the computer package SMATR (Warton *et al.*, 2006), in which heterogeneity between SMA slopes is tested by a permutation test.

TABLE 2. *Effect of the leachate treatment (male, hermaphrodite, control) on the sex ratio of individuals, expressed as the proportion of males (see text for details), of* M. annua*; differences between male leachate treatment and the other treatments are indicated*

Treatment	Mean	s.e.	z-value	P (>|z|)
Male	0·32	0·03		
Hermaphrodite	0·29	0·04	0·885	0·376
Soil	0·26	0·04	−1·678	0·093

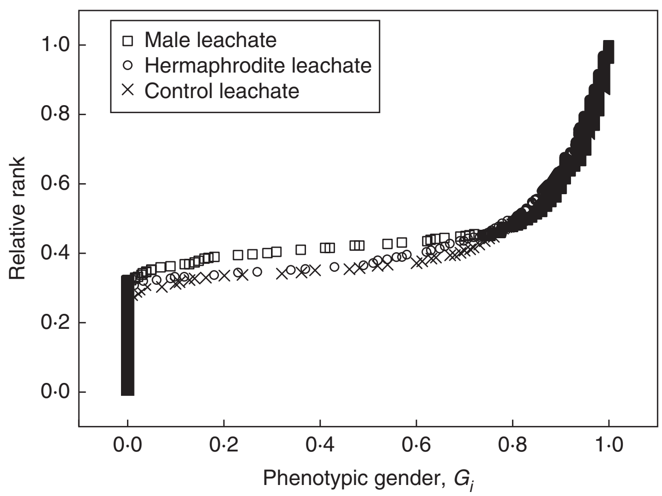

FIG. 2. The distribution of phenotypic gender in androdioecious *M. annua* growing under male, hermaphrodite and control leachate treatments, as indicated. The standardized phenotypic femaleness (G_i) is plotted against the relative rank (rank divided by sample size) for this character; $n = 300$ for male and hermaphrodite leachate, and $n = 299$ for control leachate. Plants with a G_i value of 0 and 1 are strictly male and female, respectively.

RESULTS

Effect of leachate on sex expression

The proportion of males did not differ significantly among the leachate treatments; across the experiment, 29 % of the individuals grown developed as males (Table 2). There were also no significant affects of leachate source on the distribution of phenotypic gender (Kolmogorov–Smirnov test: $P > 0·30$ for all possible combinations tested; Fig. 2).

Effect of leachate treatment on sexual dimorphism

Overall, plants displayed similar patterns of sexual dimorphism as documented previously (Hesse and Pannell, 2011*a*). Hermaphrodites allocated more biomass to total reproduction and they also had greater above-ground dry mass (Table 3, Fig. 3A, C). PC2 (inversely correlated with branch and internode lengths) was significantly greater for hermaphrodites (Table 3, Fig. 3E). These differences depended on the leachate treatments (Table 3, interaction sex × leachate): application of the male leachate elicited the smallest difference in above-ground dry mass between males and hermaphrodites, whereas the greatest difference was found for plants treated by the control leachate (Fig. 3C).

Effect of leachate on reproductive effort

Individuals treated with hermaphrodite leachate allocated less to reproduction than those treated with male or control leachates (Table 3, Fig. 3A). In particular, males allocated less biomass to reproduction under the hermaphrodite leachate than under the control leachate ($F_{2,245} = 3·057$, $P = 0·048$; Fig. 4A). Leachate treatment did not affect the allocation of biomass to male reproductive function in hermaphrodites ($F_{2,620} = 0·0296$, $P = 0·971$; Fig. 4B). However, hermaphrodites that received the control leachate allocated significantly more biomass towards female flowers and fruits than those that received male leachate ($F_{2,620} = 2·997$, $P = 0·051$; Fig. 4B). Overall, the allocation to pollen by hermaphrodites

TABLE 3. *Results of the linear mixed-effect models (lme) for the effect of the leachate treatment (male, hermaphrodite, control) on total reproductive effort (TRE), height, above-ground dry mass (Above DM) and for morphological traits (PC1 and PC2) of males and hermaphrodites of* M. annua

		TRE (879)		Height (879)		Above DM (879)		PC1 (868)		PC2 (868)	
	d.f.	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Sex	1	4·96	**0·026**	0·221	<0·638	184	**< 0·0001**	0·0106	0·918	10·2	**0·0015**
Leachate	2	4·78	**0·009**	26·5	**< 0·0001**	231	**< 0·0001**	37·9	**< 0·0001**	13·7	**< 0·0001**
Sex × Leachate	2	1·61	0·201	2·51	0·0818	3·87	**0·0213**	1·71	0·182	2·18	0·114

Block was included in the analyses as a random variable (not shown) and sex, leachate and their interaction were treated as fixed factors. Error degrees of freedom are shown in parentheses in the column headings. Values significant at $P < 0·05$ are shown in bold.

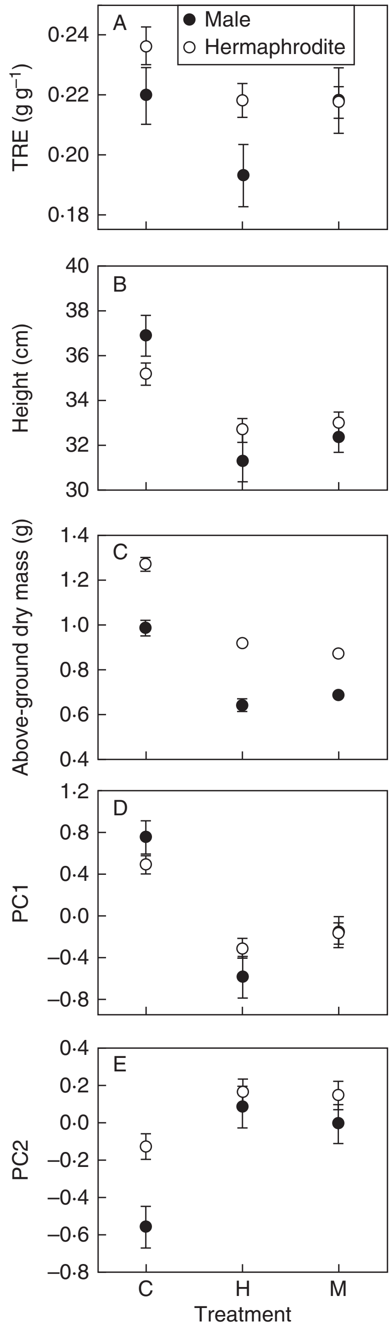

FIG. 3. Mean values (± s.e.) of (A) total reproductive effort (TRE), (B) height, (C) above-ground dry mass, (D) PC1 and (E) PC2 for males and hermaphrodites (as indicated) of *M. annua* as a function of the leachate treatment (C, control; H, hermaphrodite; M, male).

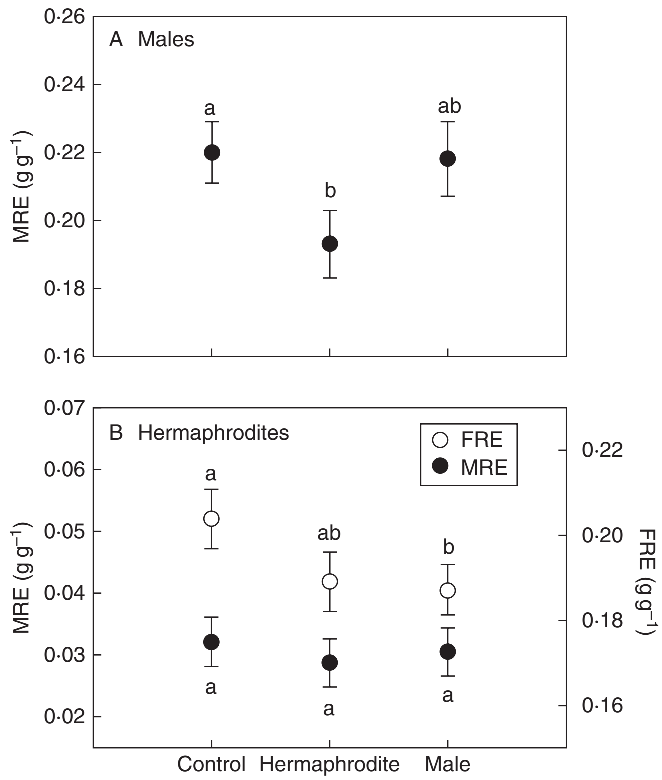

FIG. 4. Mean values (± s.e.) of (A) male reproductive effort for males (MRE) and (B) male and female reproductive effort (FRE) for hermaphrodites as a function of the leachate treatment (control, hermaphrodite and male). Different letters indicate a significant difference ($P < 0{\cdot}05$) in comparisons between the different treatments within each specific variable (FRE and MRE).

relative to that by males was similar in all three leachate treatments ($F_{2,28} = 0{\cdot}081$, $P = 0{\cdot}923$). Similarly, the amount of biomass allocated to peduncles did not differ among leachate treatments (for hermaphrodites: $F_{2,275} = 0{\cdot}115$, $P = 0{\cdot}892$; for males $F_{2,245} = 2{\cdot}64$, $P = 0{\cdot}074$).

Effect of leachate on growth and morphological traits

Leachate treatment had a significant effect on all growth, morphological and leaf-size-related traits. In particular, compared with the male and hermaphrodite leachate treatments, the control leachate significantly increased plant height, above-ground dry mass and PC1 and decreased PC2 (Table 3; Fig. 3B–E).

SMA analysis found a significant relationship between total reproductive dry mass and above-ground dry mass for all treatments (Fig. 5). Male and hermaphrodite treatments did not differ in slope (*b*) between total reproductive dry mass and above-ground dry mass (common $b = 1{\cdot}59$, test statistic = $0{\cdot}750$, $P = 0{\cdot}413$). However, there was a significant difference between slopes of male and control leachate treatments (common $b = 1{\cdot}73$, test statistic = $5{\cdot}33$, $P = 0{\cdot}022$), and those of hermaphrodite and control leachate treatments (common $b = 1{\cdot}68$, test statistic = $10{\cdot}8$, $P = 0{\cdot}002$), with plants treated with the control leachate having a larger increase in total reproductive dry mass with the same increase in above-ground dry mass than plants treated with male or hermaphrodite leachates.

Reproductive effort of males and hermaphrodites increased with above-ground dry mass under the male leachate treatment ($r^2 = 0{\cdot}020$, $P = 0{\cdot}015$) but not for hermaphrodite ($r^2 = 0{\cdot}012$, $P = 0{\cdot}063$) or control leachate ($r^2 = 0{\cdot}006$, $P = 0{\cdot}191$). We also detected differences among slopes (common slope = -4, test = $12{\cdot}1$, $P = 0{\cdot}004$), between male and hermaphrodite leachate (common slope = $-3{\cdot}91$, test = $11{\cdot}3$, $P = 0{\cdot}003$) and between control and hermaphrodite leachate (common slope = $-3{\cdot}77$, test = $6{\cdot}11$, $P = 0{\cdot}017$).

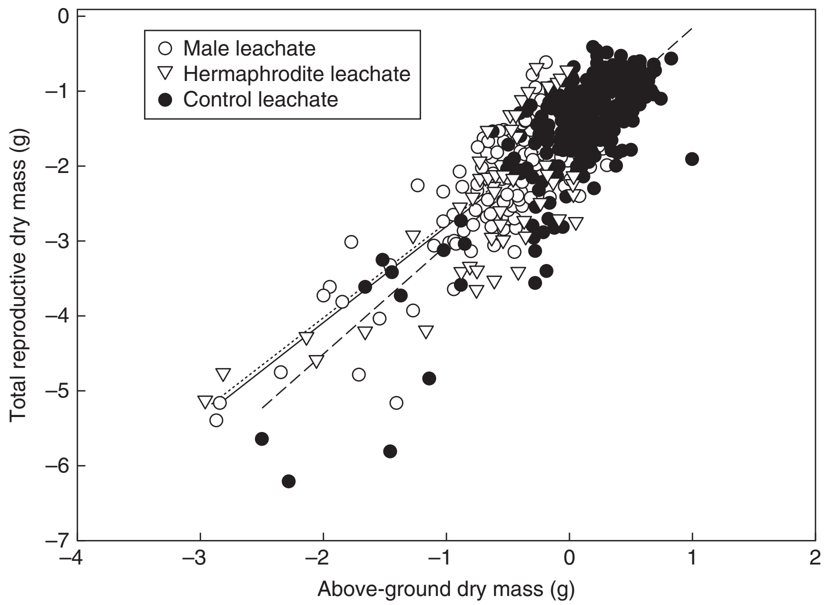

FIG. 5. Relationship between total reproductive dry mass and above-ground dry mass (ln-transformed) of plants watered with male, hermaphrodite and control leachate (as indicated). Equations of the linear regression as estimated by standardized major axis are: male, $\ln(y_1) = 1{\cdot}63 - 1{\cdot}40\ln(y_2)$; hermaphrodite, $\ln(y_1) = 1{\cdot}56 - 1{\cdot}47\ln(y_2)$; and control, $\ln(y_1) = 1{\cdot}83 - 1{\cdot}63\ln(y_2)$; y_1 and y_2 are total reproductive and above-ground dry mass, respectively.

DISCUSSION

Communication and sex choice in M. annua*?*

Our experiment found no evidence for the hypothesis that individuals of androdioecious *M. annua* choose their gender in response to soil-borne cues that might be released into the rhizosphere by neighbours: sex ratios did not differ among experimental treatments in which individuals were watered with leachate collected from pots containing conspecific males or hermaphrodites relative to those containing only soil. If individuals of androdioecious *M. annua* alter their sex expression in response to neighbours, as suggested by the density-dependent response of the sex ratio found by Pannell (1997*a*, *b*), then it is not clear that they do this by means of semiochemicals in the rhizosphere.

It is possible that our leachate treatments did affect sex expression of *M. annua*, but that our experiment was too weak to detect the difference. With the number of individuals sown per treatment (300) and the experiment-wide sex ratio observed (29 %), our analysis would only have picked up deviations in the proportion of males greater than 13 %. Our experiment ought thus to have been powerful enough to detect the differences among plant density treatments observed by Pannell (1997*b*). Of course, we did not manipulate density in our experiment here, and our leachate treatments might have been poor proxies for density (three individuals per pot), even if density is indeed detected by individuals of *M. annua* by way of chemical communication between roots. We assume that signals (if present) would be more likely to act at high density. This would then contrast with the signal used to determine sex in homosporous ferns such as *Ceratopteris richardii*, in which gametophytes develop as males with greater frequency at high density and the male frequency also responds positively to the simple presence of antheridiogen in the substrate on which gametophytes develop (Banks, 1997). It is possible that *M. annua* individuals communicate with one another via the exchange of volatile compounds above ground, as has been found for plants that respond to signals produced by neighbours attacked by herbivores (reviewed by Heil and Karban, 2010); our experiment would not have detected such above-ground communication.

In the wild, it is common to find *M. annua* seedlings that are evidently much younger than others in the same stand, but most germination typically occurs in a single flush. Our experimental application of leachate from 5-week-old seedlings to younger plants at the stage of germination and early growth is thus not particularly realistic. If signalling occurs among individuals of *M. annua* via chemicals released and perceived only by plants at early stages of growth soon after germination, our experiment will have missed evidence for it. We believe that this possibility is unlikely, because Pannell (1997*b*) found evidence for sex change quite late in plant development. Nevertheless, it would pay to repeat our experiment by passing leachate through pots with only very young seedlings to verify this. Of course, in such an experiment, it would not be possible to test for the possibility that males and hermaphrodites behave differently as potential signal producers, because separating male and hermaphrodite individuals at the germination stage is not possible.

Another possible explanation for our failure to detect an effect of leachate on the sex expression of individuals in our experiment is that sex choice was simply absent in the population we sampled. Pannell (1997*a*, *b*) found a greater frequency of males with density both in the field and in a manipulative experiment for a population located in Seville in southern Spain. This population is no longer extant. Our experiment here used seeds from a population near Fez in Morocco in which hermaphrodites are known to be particularly plastic in their sex allocation. Given the enormous variation in sex expression among populations of *M. annua*, it is however possible that the Moroccan population we sampled differs from the population sampled by Pannell (1997*a*, *b*) in terms of plants' abilities to switch between male and hermaphroditic developmental pathways. In retrospect, this possibility seems plausible. *M. annua* populations around Seville vary greatly in their male frequencies, often lacking males altogether, whereas male frequencies in those around Fez tend to be uniformly high. The advantages of assessing local mating prospects in terms of mate composition would thus seem to be greater around Seville than around Fez. It would thus be worthwhile to explore variation in the capacity for sex choice among populations of *M. annua.*

Leachate effects of growth, morphology and sex allocation

Our leachate treatments had significant effects on the total reproductive allocation and the growth and morphology of *M. annua* individuals. Almost certainly, these effects were due to a simple difference among the leachates in terms of the nutrients they were carrying. In particular, the greater growth of plants watered with leachate collected from the control pots is consistent with the possibility that these simply delivered more nutrients, presumably leached out from the compost used, than pots containing males or hermaphrodites, which presumably used much of the available nutrients in the compost before it could be leached out. Plants receiving the control leachate also allocated more to reproduction, consistent with the idea of large plants having a greater available 'budget' than smaller plants (Klinkhamer *et al.*, 1997).

The magnitude of sexual dimorphism was also affected by the leachate treatment: hermaphrodites had greater size than males, but the difference was smaller for plants growing under the male leachate treatment than under the hermaphrodite or control treatment. Decreased size differences between the sexes have been previously reported to occur in response to resource-poor conditions (Hesse and Pannell, 2011*a*, *b*). The somewhat different effects on growth of the male versus hermaphrodite leachate treatments is also consistent with the observation of a different use of soil resources by males and females in dioecious *M. annua* (Sánchez-Vilas and Pannell, 2010). The evolutionary significance of plasticity in growth and sex allocation of individuals of *M. annua* as a function of resource availability have been discussed at length elsewhere (Dorken and Barrett, 2004; Pannell *et al.*, 2008). Resource-dependent sex allocation is common in plants and has probably played a role in the evolution of combined versus separate sexes, with a tendency towards a greater separation of the sexes for plants growing under poorer conditions (Darwin, 1877; see Case and Barrett, 2004, and references therein; reviewed by Delph and Wolf, 2005).

Concluding remarks

Our experiment has provided no evidence that root–root communication via chemicals that can be leached out of the rhizosphere by overwatering influences sex choice in androdioecious *M. annua*. It is possible that an experiment using plants sampled elsewhere would have yielded different results. It would thus be valuable to assess the extent to which individuals of *M. annua* vary among populations in their capacity for density-dependent sex choice. The identification of populations that show such sex choice, like that found by Pannell (1997*a*, *b*), might then be followed up by experiments such as the one described here to determine the mode of interaction among individuals. Finally, our finding that leachate from pots containing plants of different genders, or containing plant-free soil, led to differences in growth and allocation seems simply to reflect differences in the nutrients delivered by the leachates. These results are similar to, if more subtle than, those from experiments on *M. annua* in which nutrients were manipulated directly (Harris and Pannell, 2008; Hesse and Pannell, 2011*a*, *b*). They thus confirm the sensitivity of individuals of *M. annua* to local environmental differences in their patterns of growth and reproduction.

ACKNOWLEDGEMENTS

We thank to Gerardo Albela for help in measuring sex allocation. J.S.V. was supported by a postdoctoral fellowship from Fundación Caixa Galicia (Spain).

LITERATURE CITED

Andersson M. 1983. On the function of conspicuous seasonal plumages in birds. *Animal Behaviour* **31**: 1262–1263.

Andersson M. 1994. *Sexual selection*. Princeton, NJ: Princeton University Press.

Baldwin IT, Halitschke R, Paschold A, von Dahl CC, Preston CA. 2006. Volatile signaling in plant–plant interactions: "Talking trees" in the genomics era. *Science* **311**: 812–815.

Banks JA. 1997. Sex determination in the fern *Ceratopteris*. *Trends in Plant Science* **2**: 175–180.

Bates D, Maechler M, Dai B. 2008. *lme4: Linear mixed-effects models using S4 classes*. R package version 0.999375-28. http://lme4.r-forge.r-project.org/

Case AL, Barrett SCH. 2004. Environmental stress and the evolution of dioecy: *Wurmbea dioica* (Colchicaceae) in Western Australia. *Evolutionary Ecology* **18**: 145–164.

Darwin C. 1871. *The descent of man, and selection in relation to sex*. London: John Murray.

Darwin C. 1877. *The different forms of flowers on plants of the same species.* London: Murray.

Delph LF, Wolf DE. 2005. Evolutionary consequences of gender plasticity in genetically dimorphic breeding systems. *New Phytologist* **166**: 119–128.

Dorken ME, Barrett SCH. 2004. Phenotypic plasticity of vegetative and reproductive traits in monoecious and dioecious populations of *Sagittaria latifolia* (Alismataceae): a clonal aquatic plant. *Journal of Ecology* **92**: 32–44.

Dorken ME, Pannell JR. 2008. Density-dependent regulation of the sex ratio in an annual plant. *The American Naturalist* **171**: 824–830.

Eppley SM, Pannell JR. 2007. Density-dependent self-fertilization and male versus hermaphrodite siring success in an androdioecious plant. *Evolution* **61**: 2349–2359.

Haig D, Westoby M. 1988. Sex expression in homosporous ferns: an evolutionary perspective. *Evolutionary Trends in Plants* **2**: 111–120.

Harris MS, Pannell JR. 2008. Roots, shoots and reproduction: sexual dimorphism in size and costs of reproductive allocation in an annual herb. *Proceedings of the Royal Society B, Biological Sciences* **275**: 2595–2602.

Heil M, Karban R. 2010. Explaining evolution of plant communication by airborne signals. *Trends in Ecology and Evolution* **25**: 137–144.

Heil M, Kost C. 2006. Priming of indirect defences. *Ecology Letters* **9**: 813–817.

Hesse E, Pannell JR. 2011*a*. Sexual dimorphism in androdioecious *Mercurialis annua*, a wind-pollinated herb. *International Journal of Plant Sciences* **172**: 49–59.

Hesse E, Pannell JR. 2011*b*. Sexual dimorphism in a dioecious population of the wind-pollinated herb *Mercurialis annua*: the interactive effects of resource availability and competition. *International Journal of Plant Sciences* **107**: 49–59.

Karban R, Baldwin IT, Baxter KJ, Laue G, Felton GW. 2000. Communication between plants: induced resistance in wild tobacco plants following clipping of neighboring sagebrush. *Oecologia* **125**: 66–71.

Klinkhamer PGL, de Jong TJ, Metz H. 1997. Sex and size in cosexual plants. *Trends in Ecology and Evolution* **12**: 260–265.

Krebs JR. 1976. Fish that change sex. *Nature* **259**: 10–11.

Lloyd DG. 1980. Sexual strategies in plants. III. A quantitative method for describing the gender of plants. *New Zealand Journal of Botany* **18**: 103–108.

Lloyd DG, KS Bawa. 1984. Modification of the gender of seed plants in varying conditions. *Evolutionary Biology* **17**: 255–338.

Moran MD. 2003.. Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos* **100**: 403–405.

Nakagawa S. 2004. A farewell to Bonferroni: the problems of low statistical power and publication bias *Behavioral Ecology* **15**: 1044–1045.

Pannell J. 1997*a*. Variation in sex ratios and sex allocation in androdioecious *Mercurialis annua*. *Journal of Ecology* **85**: 57–69.

Pannell J. 1997*b*. Mixed genetic and environmental sex determination in an androdioecious population of *Mercurialis annua*. *Heredity* **78**: 50–56.

Pannell J. 1997*c*. Widespread functional androdioecy in *Mercurialis annua* L. (Euphorbiaceae). *Biological Journal of the Linnean Society* **61**: 95–116.

Pannell JR, Dorken ME, Pujol B, Berjano R. 2008. Gender variation and transitions between sexual systems in *Mercurialis annua* (Euphorbiaceae). *International Journal of Plant Sciences* **169**: 129–139.

R Development Core Team. (**2009**). *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. www.r-project.org.

Sánchez-Vilas J, Pannell JR. 2010. Differential niche modification by males and females of a dioecious herb: extending the Jack Sprat effect. *Journal of Evolutionary Biology* **23**: 2262–2266.

Shulaev V, Silverman P, Raskin I. 1997. Airborne signalling by methyl salicylate in plant pathogen resistance. *Nature* **385**: 718–721.

Warton DI, Wright IJ, Falster DS, Westoby M. 2006. Bivariate line-fitting methods for allometry. *Biological Reviews* **81**: 259–291.

Wilson K, Hardy ICW. 2002. Statistical analysis of sex ratios: an introduction. In: **Hardy ICW**. ed. *Sex ratios: concepts and research methods*. Cambridge, UK: Cambridge University Press, 48–92.