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Enhanced leaky sex expression in response to pollen limitation in the dioecious plant *Mercurialis annua*

Guillaume G. Cossard Ph.D 🄳 | John R. Pannell 🄳

Department of Ecology and Evolution, Biophore Building, University of Lausanne, Lausanne, Switzerland

Correspondence
John R. Pannell, Department of Ecology and Evolution, Biophore Building, University of Lausanne, CH-1015 Lausanne, Switzerland.
Email: john.pannell@unil.ch

Present address
Guillaume G. Cossard, Algal Genetics Group, Integrative Biology of Marine Organisms Department (UMR8227), Station Biologique CNRS UPMC, Roscoff Cedex, France

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Abstract

In dioecious plants, males and females frequently show 'leaky' sex expression, with individuals occasionally producing flowers of the opposite sex. This leaky sex expression may have enabled the colonization of oceanic islands by dioecious plant species, and it is likely to represent the sort of variation upon which selection acts to bring about evolutionary transitions from dioecy to hermaphroditism. Although leakiness is commonly reported for dioecious species, it is not known whether it has plastic component. The question is interesting because males or females with an ability to enhance their leakiness plastically in the absence of mates would have an advantage of being able to produce progeny by self-fertilization. Here, we demonstrate that leaky sex expression in the wind-pollinated dioecious herb *Mercurialis annua* is plastically responsive to its mating context. We compared experimental populations of females growing either with or without males. Females growing in the absence of males were leakier in their sex expression than controls growing with males, producing more than twice as many male flowers. Our results thus provide a striking instance of plasticity in the reproductive behaviour of plants that is likely adaptive. We consider how females might sense their mating environment as a function of pollen availability, and we discuss possible constraints on the evolution of plasticity in sex expression when the environmental signals that individuals receive are unreliable.

KEYWORDS
androdioecy, dioecy, hermaphroditism, inconstancy, plasticity, reproductive assurance, sex allocation

1 | INTRODUCTION

Phenotypic plasticity, the expression of different phenotypes by the same genotype under different conditions, is all but ubiquitous across the tree of life and is particularly pronounced in plants (Bradshaw, 1965; Josephs, 2018; Sultan, 1987). This is likely due both to their sessile nature, which means that they cannot actively choose their habitat and must make the best of the conditions to which they are exposed, as well as to their modularity, which allows individuals to modify the phenotype of their modules (e.g., branches, inflorescences or flowers) as they are produced in response to environmental variation over time and space. Nevertheless, evidence that plastic variation in plants is adaptive remains surprisingly thin, with few clear demonstrations that the different phenotypes expressed

in different environments actually improve fitness (Hendry, 2015; Van Kleunen & Fischer, 2005; Wagner & Mitchell-Olds, 2018). Several studies have demonstrated that plant responses to shading are adaptive, with fitness benefits to individuals that produce longer internodes and achieve greater height when overtopped by or growing beside potential competitors (e.g. Dudley & Schmitt, 1995, 1996). The plastic induction of increased defence in response to herbivory has also been shown to increase individuals' fitness (e.g., Agrawal, 1998, 1999; Karban et al., 1997). Further, Baythavong (2011) and Baythavong and Stanton (2010) showed that variation in a number of morphological and phenological traits was adaptive in environments with small-scale variation in soil chemistry, and Kenney et al. (2014) showed that plasticity in water-use efficiency (WUE) conferred fitness benefits under dry conditions—though Nicotra and Davidson (2010) reported inconsistent findings in their review of plasticity in WUE among studies.

Plant reproductive traits may also be phenotypically plastic. Examples include variation in reproductive effort or reproductive allocation, which is sensitive to resource availability and competition (Weiner, 2004); sex allocation, for example in terms of the relative numbers of male versus female flowers produced by monoecious individuals, which varies with plant size and resource status (Dorken & Barrett, 2003; Pannell, 1997; Paquin & Aarssen, 2004); and floral longevity and floral display size, with plants adjusting their attractiveness to pollinators in response to the relatedness of their neighbours (Torices et al., 2018) or as a function of relative pollinator abundance and visitation rates (Harder & Johnson, 2005). This latter example is particularly interesting in the context of our study here, because it indicates the extent to which plants may alter their reproductive allocation decisions specifically in response to plant mating opportunities. Specifically, Harder and Johnson (2005) found that floral display in the hermaphroditic orchid *Satyrium longicauda* was enhanced when pollinator visitation rates were low, increasing the possibility for later pollen receipt or geitonogamous self-pollination, with likely fitness benefits. Similarly, Lopez and Dominguez (2003) found that in the monoecious plant *Begonia gracilis*, individuals whose female flowers were pollen-limited produced more male flowers than those whose female flowers enjoyed experimentally augmented pollen deposition, suggesting that plants can respond to the operational sex ratio of the population (though without demonstrating a clear effect on fitness in natural populations). In homosporous ferns, gametophytes are more likely to develop as males when females or hermaphrodites are locally abundant, a switch mediated by interplant chemical signalling (Banks, 1997).

Many angiosperms with separate sexes also show variation in sex expression. Specifically, the males and females of dioecious plants commonly display inconstant or 'leaky' sex expression, with the occasional production of a few flowers of the opposite sex (e.g., Baker, 1967; Delph, 2003; Diggle, 1991; Korpelainen, 1998; Lloyd, 1972; Lloyd & Bawa, 1984; Venkatasamy et al., 2007). Such leaky sex expression, which is more common in males than females (Delph & Wolf, 2005; Ehlers & Bataillon, 2007, though see Cossard & Pannell, 2019), has probably been important in

facilitating evolutionary transitions from dioecy to monoecy or hermaphroditism under conditions of mate limitation (Crossman & Charlesworth, 2014; Ehlers & Bataillon, 2007; Käfer et al., 2017). It is plausible that leaky dioecy may be adaptive by assuring reproductive success under pollen- or mate-limited conditions, for example during the colonization of oceanic islands that are enriched for dioecious plant species (Baker, 1965; Pannell et al., 2015; Stebbins, 1965; Sultan & Spencer, 2002). However, leaky sex expression can be elicited by external stimuli such as temperature, drought, simulated herbivory or exogenous hormone application (Delph & Wolf, 2005; Golenberg & West, 2013; Korpelainen, 1998; Kuhn, 1939; Westergaard, 1958), none of which suggest an obvious adaptive function. Importantly, there appears to be no empirical support to date for the possibility that leaky sex expression might be prompted by pollen or mate limitation, which would be more plausibly adaptive.

Here, we demonstrate that leaky sex expression in the dioecious, wind-pollinated annual herb *Mercurialis annua* is plastic and that the expression of enhanced leakiness under conditions of altered mate availability is likely adaptive. Dioecious *M. annua* has an XY system of sex determination in which the Y chromosome has a mildly degenerate nonrecombining region (Li et al., 2019; Veltsos et al., 2018, 2019). Sex ratios in wild populations are typically 1:1 (Russell & Pannell, 2015). The species is strongly sexually dimorphic, with males and females differing in a number of physiological, life-history and morphological characters (Tonnabel et al., 2019; Tonnabel et al., 2019). Yampolsky (1919); Yampolsky and Yampolsky (1922) noted the presence of 'intergrades' in both sexes of *M. annua* (evidently individuals showing leaky sex expression), though Cossard and Pannell (2019) showed that females are more often leaky than males. Yampolsky (1930) and Kuhn (1939) demonstrated that leakiness in *M. annua* could be enhanced by pruning, but it was not obvious from these studies that the plastic response was adaptive rather than simply a physiological response to unaccustomed stress. Leakiness in *M. annua* females involves the production of staminate (male) flowers that are effectively identical to those on males, except that they tend to be clustered around the pistillate (female) flowers in the leaf axils, whereas males place their flowers on stalked 'peduncles'. Staminate and pistillate flowers can be distinguished very early in development, at the bud stage.

Our experiment involved growing females of *M. annua* in populations with or without males. We predicted that, under a scenario of adaptive leaky sex expression, females growing without males would be more likely to produce male flowers and would produce more of them. There are several reasons to expect that selection might have favoured a plastic leakiness in sex expression in *M. annua* in response to variation in mate availability. First, the dioecious populations of *M. annua* are known to have expanded their range recently from the eastern Mediterranean Basin into western Europe (Gonzalez-Martinez et al., 2017; Obbard et al., 2006), during which populations establishing at the colonizing front are likely to have been exposed to mate-limited conditions, but mate limitation would be ameliorated with population growth. Second, the species has a metapopulation structure and dynamic, with

frequent population turnover and substantial fluctuations in population size and sex ratio from generation to generation (Dorken et al., 2017; Eppley & Pannell, 2007); as with a range expansion, plastic leaky expression of the opposite sex would likely confer fitness during episodes of colonization, even after a range expansion ended (Golenberg & West, 2013). Third, previous work has shown that females of *M. annua* quickly become pollen-limited at low population density (Hesse & Pannell, 2011), so that plastic expression of leakiness in sparse populations would be beneficial. Fourth, inbreeding depression in western European populations is low (Eppley & Pannell, 2009), perhaps as a result of the range expansion (Gonzalez-Martinez et al., 2017; Pujol et al., 2009), so that selfing by leaky individuals under pollen limitation would seem to be particularly likely to be beneficial (Pannell, 2008; Wolf & Takebayashi, 2004). Finally, individuals in all-female populations established by leaky females would have particularly high siring success if they could respond to the absence of males by producing more pollen (Dorken & Pannell, 2009).

2 | MATERIALS AND METHODS

We established six experimental populations of dioecious *M. annua* in separate common gardens on the campus of the University of Lausanne and in gardens around the city. Three 'control' populations were established at a 1:1 sex ratio (90 males and 90 females), and three 'all-female' population comprised only females (180 females). Plants were established for the experiment from a well-mixed pool of seeds from 35 demes of a metapopulation in north-western Spain (Labouche & Pannell, 2016), ensuring that there were no genetic differences between the different treatments or replicates. Seedlings were first raised for six weeks together in a glasshouse before being transplanted into pots in their experimental plots ('populations'). Importantly, there was no difference between plants eventually assigned to the two treatments while being raised to maturity in the glasshouse; the differences were imposed only during transplant into the experimental plots, which individuals effectively allocated to plots randomly. Experimental populations were established in garden sites scattered around Lausanne and were thus widely separated. For logistical reasons, three plants were established in each of 60 pots, with three females in each pot in the all-female populations, and two males and a female alternating with two females and a male in the control populations. We included the number of males per pot in an initial analysis of our results, but found that it did not have an effect on the reproductive effort in either sex function (see Results). After 7 weeks of subsequent growth, we recorded male and female reproductive allocations and the total dry biomass of all individuals separately. Male and female reproductive allocations were measured in terms of the biomass of all-male flowers, or of all-female flowers and fruits (and seeds) found on an individual at the time of harvest. Sample sized ranges from 35 to 50 females per population. Most of these plants (192) were chosen from separate pots, but 13 pots contributed two and 18 pots contributed three plants to our

data set. In total, there were 272 females distributed among the two treatments and six plots.

We analysed the extent of leaky sex expression by females in terms of (a) the proportion of leaky females in the population (the probability that a female showed leaky sex expression) and (b) their male and female allocations. To calculate proportions, we defined a leaky female as one with any male reproductive effort (MRE; male-flower biomass divided by total above-ground biomass) greater than the 95 percentile MRE across the control populations. By this definition, an average 5% of females were identified as leaky across the three control populations. We chose the 95% threshold for our definition because it can be easy to miss one or two small male flowers on a large plant, and for coherence with previous work on leaky sex expression in *M. annua* (Cossard & Pannell, 2019). An analysis based on an absolute measure of leakiness (including all females with any male-flower production at all) yielded qualitatively similar results, as did an analysis based on adopting a leakiness threshold of 80%.

We compared the proportion of females with leaky sex expression between all-female and control populations using a generalized linear model with a binomial error distribution, and with population and pot included as random factors. We used a generalized linear mixed model to compare the male and female allocations of females between the all-female and the control populations, including treatment and above-ground plant vegetative biomass (offset, log-transformed) as fixed effects, and with population and pot declared as random effects. Results for mean allocations are presented in terms of the male or female components of reproductive effort (MRE and FRE, respectively), calculated as the male or female reproductive biomass divided by the above-ground vegetative biomass.

3 | RESULTS AND DISCUSSION

Our results provide evidence for a plastic component to the expression of sex inconstancy in *M. annua*. First, females tended to be more likely to be leaky in populations lacking males, though the different fell just short of statistical significance ($p = .058$; Figure 1a). Second, females growing in the absence of males produced on average 2.33 times more male flowers, measured in terms of MRE, than in their presence ($p = .01$; Figure 1b). We also found that the seed and fruit production by females in the all-female populations, measured in terms of FRE, was slightly lower than that of females in control populations, but the difference was not significant ($p = .19$; Figure 1c; note that the mean FRE varied significantly more among all-female populations than among the control populations; F-test for equal variances between treatment and control means: $F_{2,2} = 0.005$, $p = .01$). Neither the MRE ($p = .851$) nor the FRE of females ($p = .977$) was sensitive to the number of males sharing the same pot, nor was the probability of being leaky ($p = .11$).

Our study demonstrates that leaky sex expression in *M. annua* has an important plastic component. We do not know how plants in our experiment assessed their mating prospects, but our failure to find any effect of the number of males sharing a pot with the
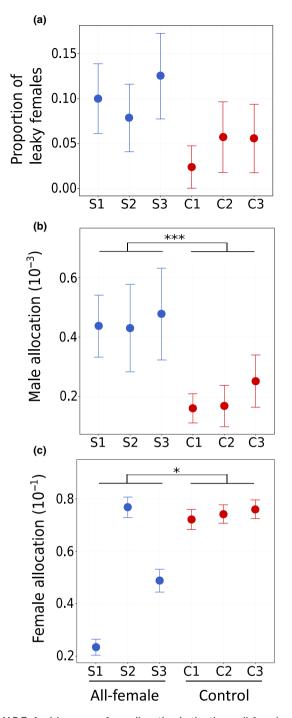
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FIGURE 1 Measures of sex allocation in the three all-female (S1–S3, blue) and the three control populations (C1–C3, red). (a) Proportion of females with leaky sex expression, (b) male allocation, calculated in terms of the male reproductive effort of females as the biomass of male flowers divided by the above-ground vegetative biomass per plant, and (c) female allocation, calculated in terms of the female reproductive effort of females as the biomass of female flowers and fruits divided by the above-ground vegetative biomass per plant. Means and standard errors are plotted.**$0.001 < p < .01$

target females suggests that it was probably not through an ability to distinguish between the presence or absence of a male neighbour Rather, we speculate that plasticity in the leakiness of *M. annua*

females was sensitive to mating opportunities directly, for example through perception of the pollination (or its absence) of female flowers on the plant—though this speculation requires formal testing. As summarized in the Introduction, leaky sex expression is a common feature of the reproduction of dioecious plants (Cossard & Pannell, 2019; Ehlers & Bataillon, 2007; Korpelainen, 1998; Lloyd & Bawa, 1984). While it is too early to speculate on the frequency of plasticity in leaky sex expression in dioecious plants generally, mate availability likely varies substantially in natural plant populations, so that leaky sex expression is a trait that dioecious populations might often evolve.

Mercurialis annua is a ruderal species that occupies disturbed habitat, and previous work has shown that its populations are subject to substantial fluctuations in population size and population turnover, with local extinctions and colonization by seed dispersal being key features of the species' ecology (Dorken et al., 2017; Eppley & Pannell, 2007; Pannell, 1997). In sparse populations of *M. annua*, female reproduction may be strongly pollen-limited (Hesse & Pannell, 2011), and a capacity to produce male flowers and to self-fertilize under these conditions is likely to be adaptive, especially as inbreeding depression is low (Eppley & Pannell, 2009). It is thus plausible that plasticity in leaky sex expression has evolved and/or has been maintained under conditions of fluctuating population size and density in metapopulations. *Mercurialis annua* came to occupy its broad range in western Europe via a recent range expansion from the eastern Mediterranean Basin (Gonzalez-Martinez et al., 2017; Obbard et al., 2006), and the repeated demographic bottlenecks that occurred during the range expansion may also have favoured the maintenance of plasticity in leaky sex expression.

Ultimately, plasticity in sex expression in dioecious *M. annua* should be maintained as long as its benefits outweigh its costs. Potential costs of plasticity, which might constrain its evolution (reviewed in Van Kleunen & Fischer, 2005), include physiological costs to the plant of acquiring accurate information about the state of the environment, and costs of maintaining a sensitivity to environmental cues. Despite considerable effort, however, it has been difficult to find any evidence for such costs (Auld et al., 2010). Rather, it seems more likely that the evolution of plasticity is held in check by so-called 'limits' associated with its deployment, such as the penalty paid by individuals that respond incorrectly to an unreliable signal, or the disadvantage of having to wait until a signal is perceived before expression of an appropriate phenotype (Auld et al., 2010; Murren et al., 2015). We do not know which of these limits might apply to plasticity in leaky sex expression, but both seem plausible. In short-lived species like *M. annua*, or in species with a short reproductive season, there might be strong disadvantages associated with delaying leaky sex expression until mating prospects have been perceived. Yet, such delays seem inevitable if, as suggested above, the signal to which *M. annua* females are responding is the actual deposition of pollen on their stigmas, or physiological signals arising from seed-filling or fruit-set that follow pollination.

In conclusion, our study has demonstrated that leaky sex expression in *M. annua* has a substantial plastic component. Such leakiness is common in dioecious plants and likely contributes to their ability to colonize oceanic islands, where they are especially frequent (Baker, 1955; Pannell, 2015; Pannell et al., 2015). There seems little doubt that leaky sex expression will be advantageous for individuals of dioecious species during episodes of colonization. Demonstrating that *plasticity* in leakiness is advantageous and adaptive in such individuals is much harder. In the case of *M. annua*, it will require not only showing that leaky individuals have higher reproductive success in the absence of mates than nonleaky individuals, but also that nonleaky individuals have higher reproductive success than leaky individuals when mates are abundant. Such plasticity in leakiness seems most likely to be adaptive in weedy species such as *M. annua* that frequently colonize empty habitat patches, and for which mating prospects thus vary considerably over time and space.

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AUTHOR CONTRIBUTIONS

GGC and JRP conceived the study. JRP collected the data. GGC analysed the data. GGC and JRP wrote the manuscript.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data on which this paper is based have been deposited with Dryad at the following address: https://doi.org/10.5061/dryad.nvx0k6dqq

ORCID

Guillaume G. Cossard (ID) https://orcid.org/0000-0003-4633-5159
John R. Pannell (ID) https://orcid.org/0000-0002-0098-7074

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