
Biological relevance of polyploidy: ecology to genomics

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Polyploidy and the sexual system: what can we learn from *Mercurialis annua*?

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The evolutionary success of polyploidy most directly requires the ability of polyploid individuals to reproduce and transmit their genes to subsequent generations. As a result, the sexual system (i.e. the mating system and the sex allocation of a species) will necessarily play a key role in determining the fate of a new polyploid lineage. The effects of the sexual system on the evolution of polyploidy are complex and interactive. They include both aspects of the genetic system, the genetic load maintained in a population and the ecological context in which selection takes place. Here, we explore these complexities and review the empirical evidence for several potentially important genetic and ecological interactions between ploidy and the sexual system in plants. We place particular emphasis on work in our laboratory on the European annual plant *Mercurialis annua*, which offers promising scope for detailed investigations on this topic. *M. annua* forms a polyploid complex that varies in its sexual system from dioecy (separate sexes) through androdioecy (males and hermaphrodites) to functional hermaphroditism. © 2004 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2004, 82, 547–560.

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INTRODUCTION

For many species, the most important factor affecting the initial spread, and evolutionary maintenance, of a polyploid lineage is its sexual system. This is because it determines both the transmission of genes from one generation to the next and the genetic architecture upon which natural selection acts. The sexual system encompasses those aspects of a species' biology that regulate (1) the allocation of resources to male, female and ancillary functions such as pollinator attraction and reward (the sex allocation), and (2) the rates of self-fertilization and outcrossing in the population (the mating system) (Barrett, 2002).

Phenotypic models have shown that the invasion of a population by sex-allocation or mating-system modifiers depends on aspects of pollination biology and the relative fitness of selfed vs. outcrossed progeny, often

irrespective of how the phenotypic characteristics are determined genetically (Lloyd, 1975, 1983; Charnov, 1982; Zhang, 2000). The initial spread of such modifiers should therefore not depend on a species' ploidy *per se*. By contrast, the way the mating system evolves subsequent to the initial spread of a modifier may indeed depend rather strongly on the underlying genetics (e.g. Charlesworth & Charlesworth, 1978; Lande & Schemske, 1985; Lande, Schemske & Schultz, 1994; Schultz, 1999) and thus may differ under different ploidy backgrounds. It is thus important to distinguish between the evolution of a new or modified sexual system in a population, and its subsequent maintenance through time.

A principal aim of this paper is to explore the complex interactions we expect to find between the evolution of polyploid lineages and their sexual systems and to review relevant studies of bearing on this issue. We distinguish between the direct effects of polyploidization on the reproductive system, and the subsequent evolution of the sexual and genetic systems once poly-

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ploid populations have become established. The former effects can generally only be studied through analysis of very recently polyploidized lineages in nature, or of artificially induced polyploids (Ramsey & Schemske, 2002), whereas the latter may be addressed through replicated comparisons of established polyploid lineages with their diploid progenitors. For clarity of discussion, we also discriminate between the ecological and genetic interactions between polyploidy and the sexual system. Although these two aspects are interrelated, it is useful to distinguish the evolution of the genetic system (including the genetic load) under polyploidy from the often indirect effects of genome duplication on the sexual system through changes in a species' ecological context.

Our other major aim is to summarize past and ongoing work on the *Mercurialis annua* species complex, which displays unusually broad variation in both its sexual system and its ploidy (Durand & Durand, 1992). Although a taxonomically broad comparative analysis of ploidy and the mating system may one day prove revealing (but see Charlesworth, 2001), the sort of data required for such studies are still very limited. A great deal therefore remains to be learnt from detailed analysis of individual species or genera, such as *Mercurialis*, within which appropriate variation is displayed.

MERCURIALIS ANNUA AS A MODEL SYSTEM

Mercurialis annua L. (Euphorbiaceae) is a wind-pollinated annual plant that occupies ruderal and roadside habitats throughout central and western Europe and around the Mediterranean Basin (Tutin *et al.*, 1964). It is naturalized in North America, the Caribbean, South Africa and Japan (Durand, 1963). In mesic climates, plants flower throughout the year, but the species is a winter annual in the Mediterranean region. Primary seed dispersal is ballistic, with secondary dispersal by ants (Lisci & Pacini, 1997), although seeds are also doubtless moved in soil by humans and may be blown substantial distances by wind (our pers. observ.).

There is a long history of research on *M. annua* that dates back to early investigations of sex determination and sex expression in dioecious populations by Heyer (1884), Yampolsky (1919, 1930) and Gabe (1939), and the species has continued to be a model for studies of sex determination in plants (Delaigue *et al.*, 1984; Durand & Durand, 1991; Pannell, 1997b). A firm foundation for research on the evolution of ploidy and the sexual system was established by Durand (1963) in a biosystematic study of the species complex.

The genus *Mercurialis* comprises 7–10 European species and one Asian species; all except the *M. annua* group are rhizomaceous or woody perennials (Tutin

et al., 1964; Krahenbuhl, Yuan & Kupfer, 2002). On the basis of morphology, ploidy and sexual system, Durand (1963) recognized four annual species within this group: *M. huetii* Hanry, *M. annua* L., *M. ambigua* L. fil. and *M. monoica* (Moris) Durand. Of these, *M. huetii* is quite distinct, whereas it is difficult to identify diagnostic morphological characters amongst the others (Durand & Durand, 1985). Given the reported monophyly of the clade into which *M. annua*, *M. ambigua* and *M. monoica* fall (Krahenbuhl *et al.*, 2002) and the difficulty in species delimitation between them, we refer to these three putative taxa together as *M. annua* in the broad sense.

Diploid populations of *M. annua* ($2n = 16$) range from Israel throughout central and western Europe into southern France and northern Spain; these populations are uniformly dioecious (Durand, 1963; Fig. 1). In northern Spain, diploid populations give way to hexaploid populations ($2n = 48$) across two abrupt transitions on the Mediterranean and Atlantic coasts. In Catalonia in the east, this transition coincides with a shift from dioecy to self-compatible monoecy, whereas in Galicia in the west, dioecy gives way to a narrower band of monoecy, followed by androdioecy further south (i.e. the co-occurrence of males with functional hermaphrodites). South of these transitions, hexaploid *M. annua* populations are variously monoecious or androdioecious (Durand, 1963; Fig. 1). In northern Morocco, around Fes and Meknes, hexaploid populations are subdioecious, with males and females co-occurring with female-biased monoecious plants. Tetraploid populations of *M. annua* ($2n = 32$) are found on the coast in central western Morocco, where individuals are generally monoecious, and higher ploidy levels (up to $12x$) are found further east in north Africa and on the Mediterranean islands of Corsica and Sardinia; these populations are all monoecious (Durand, 1963; Fig. 1).

In the *M. annua* species complex, there is evidently a correspondence between polyploidy and monoecy. This raises the question of whether monoecy is a direct consequence of polyploidization, whether polyploidy has only been able to arise in a selfing lineage, or whether polyploidy has allowed selection on the sexual system to favour monoecy subsequent to genome duplication. Although ploidy and the sexual system are broadly confounded in *M. annua*, variation in the sexual system among hexaploid populations in particular offers an opportunity to conduct replicated comparisons between sexual systems in isolation of ploidy effects.

In short, *M. annua* presents valuable material with which to address questions concerning the establishment and evolution of polyploid races with contrasting sexual systems. Its notable features can be summarized as: (1) broad correspondence between polyploidy

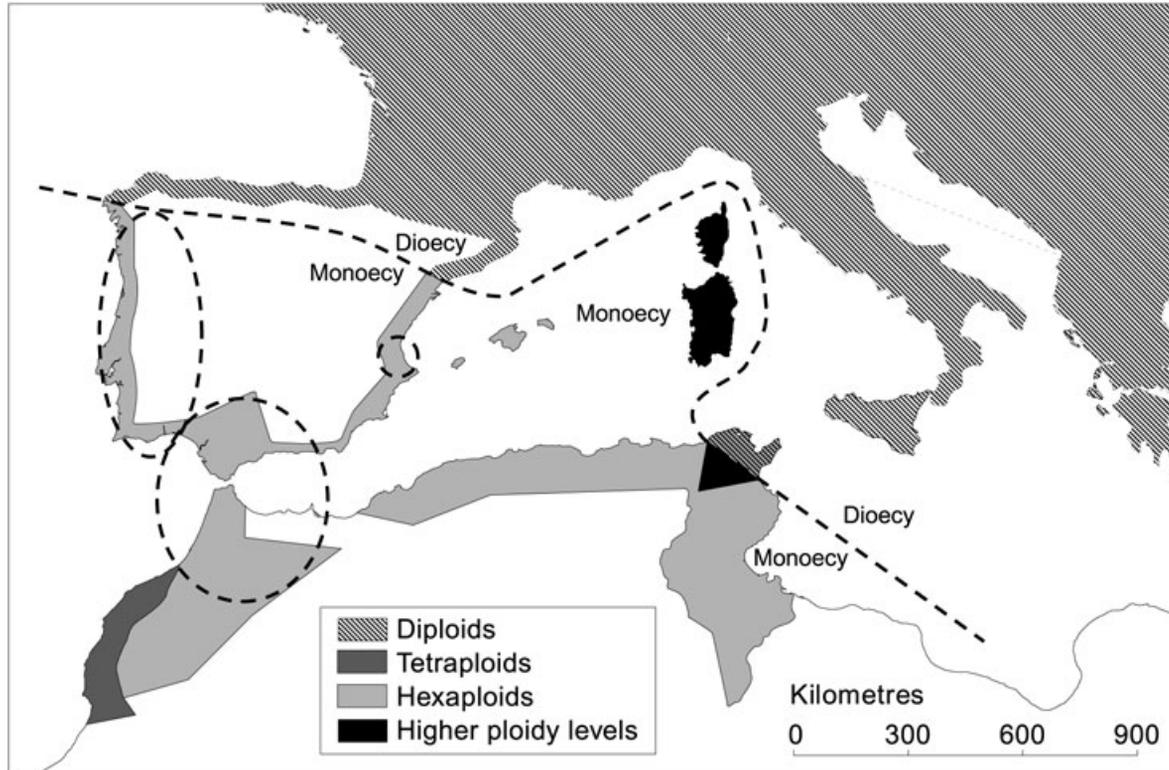


Figure 1. The distribution of different ploidy levels in the *Mercurialis annua* complex around the Mediterranean Basin. The dashed ellipses designate regions in which androdioecious populations are found. Map modified after Durand (1963).

and monoecy, derived from dioecy; (2) within-ploidy variation in the sexual system among hexaploid populations; and (3) two effectively independent diploid–hexaploid contact zones in northern Spain. Being a fast-growing annual, the plant is easy to manipulate under controlled conditions, and natural populations are accessible and highly abundant in the field. Artificial neo-polyploids can be produced under laboratory conditions (Durand, 1963), monoecious plants can be readily self-fertilized by isolating them in pollen-proof growth boxes (Pannell, 1997c) and the self-fertilization of unisexual plants is possible by altering their gender expression through the exogenous application of phytohormones (Durand & Durand, 1991).

HYPOTHESES FOR THE EVOLUTION OF THE SEXUAL SYSTEM IN *M. ANNUA*

Given the universal occurrence of dioecy in the other species of *Mercurialis*, and particularly in diploid *M. annua*, there is little doubt that monoecy in the polyploid populations is a derived trait (Krahenbuhl *et al.*, 2002). Whether androdioecy and subdioecy are derived from dioecy or secondarily from monoecy is not yet known, but it seems clear that the genetic basis for male and female floral and inflorescence development

has been conserved throughout the species complex and genus. Males in diploid and hexaploid populations uniformly disperse their pollen from staminate flowers held on erect pedunculate inflorescences, whereas pistillate flowers are typically borne on sessile axillary pedicels. In monoecious plants, staminate flowers usually cluster around a single pistillate flower in each leaf axil, although we have occasionally found monoecious hexaploid populations that additionally have pedunculate inflorescences similar to those of males (see also Durand, 1963). It therefore seems most likely that monoecious individuals of *M. annua* are effectively modified females. The same conclusion has been reached for the hermaphrodites of other plants and animals in which androdioecy is derived from dioecy (reviewed in Pannell, 2002).

Androdioecy is a particularly rare sexual system in plants, and its evolution and maintenance was until recently difficult to explain (Charlesworth, 1984; Pannell, 2002). A key prediction made by theoretical models is that males must enjoy at least twice the siring success of hermaphrodites. This condition is most likely to be met if male pollen production is high relative to that of hermaphrodites, and if the hermaphrodite selfing rate is low (Lloyd, 1975; Charlesworth & Charlesworth, 1978; Charlesworth, 1984). Pollen pro-

duction in *M. annua* is 4–10 times higher in males than in monoecious individuals (Pannell, 1997b, c), but the species is self-compatible (Durand, 1963), and patterns of sex allocation in monoecious populations are consistent with a history of selection under repeated bouts of inbreeding (J. R. Pannell, unpubl. data). Similarly, other androdioecious plants and animals are able to self and may experience selfing rates as high as unity in the absence of males (Pannell, 2002). This would seem to be inconsistent with the prediction of high outcrossing rates for the evolution and maintenance of males.

Studies of variation in sex allocation in *M. annua* conducted in the mid-1990s (Pannell, 2002) inspired a reappraisal of theoretical models for androdioecy. In particular, apart from the importance of recognizing dioecy rather than hermaphroditism as the ancestral trait (see also Fritsch & Rieseberg, 1992), the population structure and demography of a ruderal weed such as *M. annua* suggest selection on the sexual system at both the population and the metapopulation levels

(Pannell, 2001; J. R. Pannell, unpubl. data). The most likely hypothesis for the maintenance of androdioecy in *M. annua*, and indeed in several other species (reviewed in Pannell, 2002), is that functional self-compatible hermaphroditism evolved from dioecy and is maintained by selection for reproductive assurance at the metapopulation level as a result of repeated bouts of mate limitation during colonization (Pannell, 2001; see Fig. 3). These functionally hermaphroditic populations will be female-biased in their sex allocation as a result of selection under self-fertilization (Hamilton, 1967; Lloyd, 1987). With local demographic growth and a concomitant reduction in the selfing rate following colonization, populations become susceptible to the invasion and spread of males, which can be maintained at the metapopulation level if gene flow among populations is sufficiently high and local extinction rates are low (Fig. 2). The observed metapopulation structure, and the high among-population variation in male frequencies in several androdioecious species, appear to conform with this model.

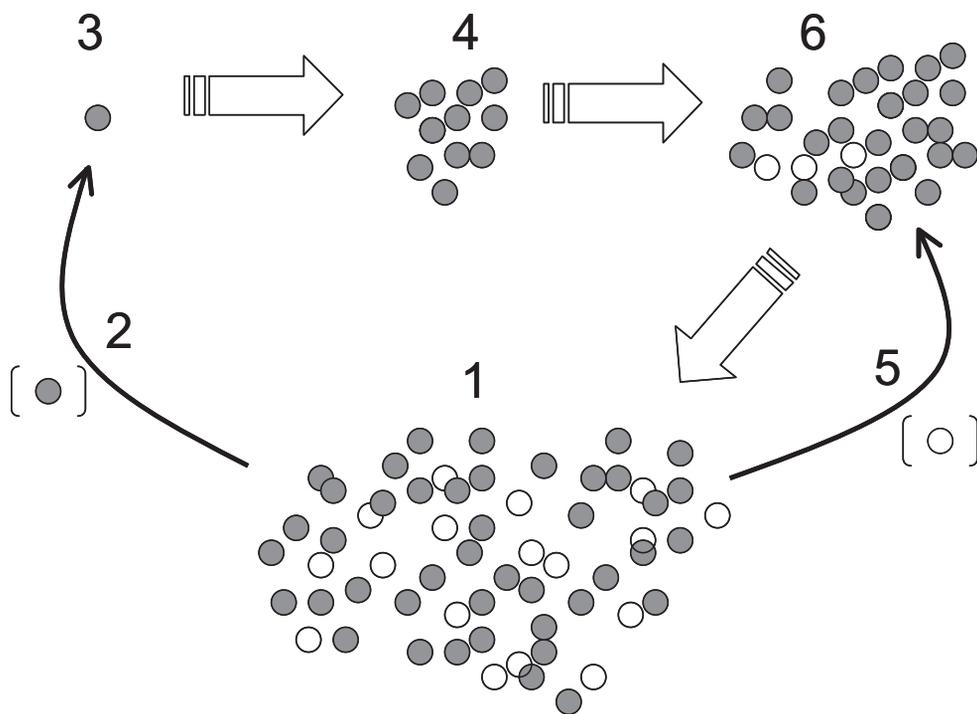


Figure 2. A graphical depiction of a model for the maintenance of androdioecy in a metapopulation. Males and hermaphrodites are symbolized by open and closed circles, respectively; block arrows represent population growth; simple arrows represent dispersal. According to the model, established androdioecious populations (1) disperse male and hermaphrodite propagules across the metapopulation (2 and 5). Only hermaphrodites can establish populations as sole colonizers due to reproductive assurance (2). New populations (3) are small and sparse and are initially highly selfing (4). As populations grow, they become denser, outcrossing with neighbours becomes easier and density-dependent selfing rates decline; the increased levels of outcrossing allow males to invade, because they have greater access to ovules (5). The age structure of the metapopulation is maintained through a balance of extinction and recolonization. Inbreeding during repeated colonization events selects for female-biased sex allocation in the hermaphrodites as a result of local mate competition.

The above scenario invokes differences in demography and population structure between dioecious, androdioecious and monoecious regions occupied by *M. annua*, with more ephemeral and genetically isolated populations found toward the monoecious end of this gradient. The evolution of monoecy (and androdioecy) from dioecy also requires a shift in the reproductive and/or demographic parameters that regulate the stability of the sexual system. Because the transition from dioecy to monoecy coincides with polyploidization in *M. annua*, the effects of polyploidy need to be integrated into hypotheses regarding both the evolution of sexual systems and their longer-term maintenance. Such an integrated framework may be built around quite general considerations of the interactions between polyploidy and the sexual system.

POLYPLOIDY AND THE SEXUAL SYSTEM

Some of the expected genetic and ecological interactions brought about by polyploidization, and their direct and indirect effects on the sexual system, are depicted in Figure 3. A useful point of departure in interpreting this figure is the recognition that transitions in the sexual system depend on changes in the

context in which it is selected. From the genetic point of view, chromosome doubling may directly reduce inbreeding depression in neopolyploids, and polyploidy may modify the total genetic load maintained in the population in the longer term (Lande & Schemske, 1985; Ronfort, 1999). The genetic upheaval associated with polyploidization might also be expected to interfere with processes in which relative allele dosage is important, such as self-incompatibility mechanisms in hermaphrodites (Lewis, 1960; Stone, 2002) and sex determination in dioecious species (Westergaard, 1958). From an ecological perspective, the expansion of a species' range, or its invasion into new habitat following polyploidization, may reduce the availability of prospective mating partners or pollinators, thus giving rise to selection for reproductive assurance and the evolution of self-fertilization (Baker, 1955; Pannell & Barrett, 1998; see Brochmann *et al.*, 2004 – this issue, for empirical examples). Selfers may be more likely to create polyploid lineages by increasing the chances of union between unreduced gametes, and selfing polyploid lineages are also likely to establish more easily than out-crossing ones as they avoid fertilization by diploid progenitors and its associated fitness costs (Levin, 1975; Rodriguez, 1996a).

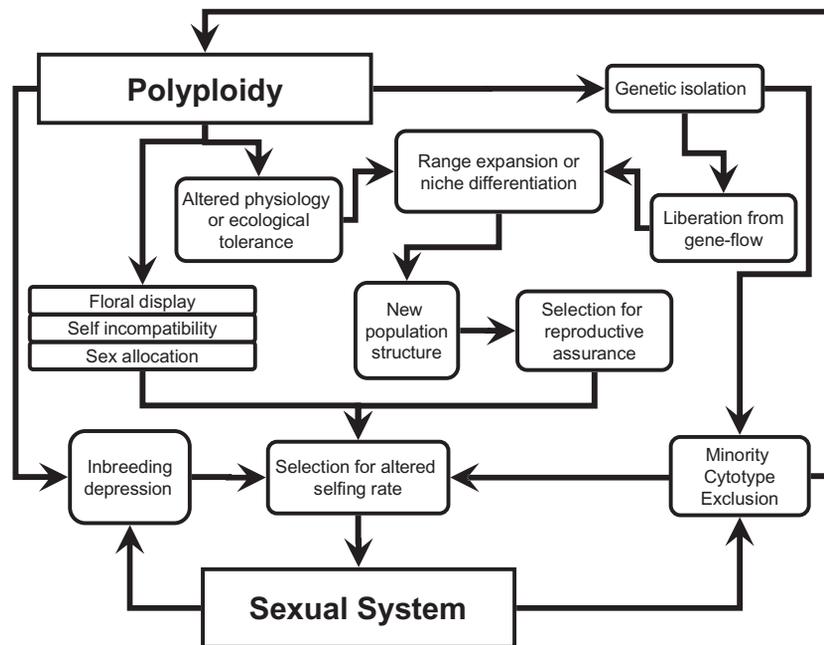


Figure 3. Hypothesized interactions between polyploidy and the sexual system. Polyploidization can have direct genetic consequences for the mating system (e.g. floral display, sex expression), which may be selected for. It can also change the genetic and ecological context in which selection acts (e.g. altered levels of inbreeding depression, and possibilities for range expansion due to altered ecophysiology and genetic isolation). Conversely, because self-fertilization increases the probability of fusion of unreduced gametes and helps to avoid the cost of minority cytotypic exclusion, the sexual system can affect the likelihood of polyploid origin and establishment. The sexual system feeds back on intermediate steps, affecting levels of inbreeding depression and the population structure.

Along with phenotypic variation or abrupt shifts in traits associated with the sexual system, which may be caused by direct genetic effects of polyploidization, inbreeding depression and reproductive assurance probably represent the two most fundamental factors affecting the evolutionary stability of the plant mating systems in general (Holsinger, 1991; Barrett & Harder, 1996; Morgan & Schoen, 1997). We address each of these three issues in the context of polyploidy below. We then consider the ways in which correlated changes in the ecology and ecophysiology of polyploids may affect the distribution of their sexual systems, and we assess the influence on cytotype distribution of ecological and genetic interactions across ploidy contact zones.

DISRUPTION OF SELF-INCOMPATIBILITY AND SEX DETERMINATION

It is well established that genome duplication can directly cause gametophytic self-incompatibility (SI) systems to break down (Lewis, 1960; Stone, 2002), because pollen grains carry multiple alleles at the SI locus (Golz *et al.*, 1999). There is also strong evidence that such self-compatible polyploid lineages persist in some families (Ross, 1981). Although the precise mechanism of this loss of function is not fully understood, it has obvious immediate implications for the evolution of the mating system. In a compelling interpretation of the association between polyploidy and dioecy in otherwise SI hermaphroditic lineages, Miller & Venable (2000) have suggested that separate sexes have often evolved as a response to selection for outcrossing following the disruption of SI mechanisms through polyploidization.

The breakdown of sex determination in dioecious lineages has similarly important implications for the mating system if this leads to the expression of both sexes in the same plant. It was an early prediction that such a breakdown would occur (e.g. Muller, 1925), and there is some limited evidence for this in neo-polyploids. For example, artificially induced autopolyploidy in dioecious *Silene* and *Rumex* gave rise to potentially selfing hermaphrodite progeny (reviewed in Westergaard, 1958). Similarly, Durand (1963) found hermaphrodite morphs in the F₂ progeny of artificial autotetraploids generated from diploid dioecious individuals of *Mercurialis annua*, with phenotypes similar to those found in natural polyploid populations. Nevertheless, despite general claims for an association between polyploidy and hermaphroditism in otherwise dioecious groups (e.g. Richards, 1997), the only examples from natural populations appear to be *M. annua* and tetraploid *Empetrum hermaphroditum*, derived from dioecious diploid *E. nigrum* (cited in Richards, 1997). More common is the reverse associa-

tion cited above between polyploidy and dioecy in lineages that were formerly SI hermaphrodites (Miller & Venable, 2000). It is quite possible that dioecy is disrupted by polyploidization and that subsequent selection on the sexual system re-establishes it over time. This is of course suggested in *M. annua*, where androdioecy and subdioecy may have evolved from monoecy in hexaploid populations following the earlier breakdown of dioecy. However, as we have hypothesized, there are reasons to believe that monoecy is in fact selectively maintained in *M. annua* over large areas of the species' range.

INBREEDING DEPRESSION

Inbreeding depression denotes the reduced fitness of self-fertilized progeny relative to the fitness of their outcrossed counterparts (Charlesworth & Charlesworth, 1987). In its absence, and under the often realistic assumption that self-fertilization uses negligible amounts of pollen, Fisher (1941) first pointed out that an outcrossing hermaphroditic population is susceptible to the invasion of self-fertilizing mutants because the latter transmit an extra copy of their genome through their seed progeny. This automatic transmission advantage to self-fertilization is counteracted by levels of inbreeding depression that exceed 0.5 (Charlesworth & Charlesworth, 1987). Populations with a sufficiently high value of inbreeding depression are thus predicted to be maintained as outcrossers unless circumstances cause inbreeding depression to drop. Under continued diploidy, this can only occur if the genetic causes of inbreeding depression are selectively lost, or 'purged', from the population (Lande & Schemske, 1985; Barrett & Charlesworth, 1991). Purging is more efficient under selfing, which causes elevated homozygosity and thus increases the opportunity for the selective loss of deleterious recessive alleles (Crnokrak & Barrett, 2002). Importantly, this process of purging implies that the mating system is stable at the extremes of completely outcrossing or the completely selfing (Lande & Schemske, 1985). On the one hand, selection in selfing populations will continue to remove deleterious genetic load as it arises through mutation, thus maintaining inbreeding depression at low levels. On the other hand, deleterious alleles can continue to accumulate at multiple loci in large outcrossing populations, because they seldom find themselves in a homozygous state when at low frequency (Lande & Schemske, 1985).

Given the importance of inbreeding depression for mating-system evolution and stability, our understanding of the interaction between polyploidy and the sexual system must depend strongly on the fitness effects of inbreeding in lineages experiencing the effects of extensive gene duplication and (potentially)

altered patterns of inheritance. It is often claimed that deleterious recessive alleles will be more effectively hidden from selection in polyploids because duplication results in a smaller proportion of homozygotes after a single round of selfing (e.g. Richards, 1997: 382). Indeed, this idea has sometimes been invoked to explain an association between selfing and polyploidy (e.g. Barrett & Shore, 1987). However, the idea of a widespread association between polyploidy and selfing, at least in angiosperms, has been questioned (Ronfort, 1999), and there has been remarkably little research on the subject to substantiate or refute it. Galloway, Etterson & Hamrick (2003) have recently drawn attention to a possible predominance of outcrossing in autopolyploids and of selfing in allopolyploids. If this is so, then it may reflect compromise of morphological outcrossing mechanisms in allopolyploids, and thus a shift to selfing. (This would be analogous to reduced outcrossing upon the breakdown of dioecy or self-incompatibility in polyploids generally.) Alternatively, selfing may be more easily maintained in allopolyploids than autopolyploids as a result of lower inbreeding depression in the former. The few existing theoretical studies concern themselves only with autopolyploids and have only dealt with populations at mutation-selection equilibrium (Bennett, 1976; Lande & Schemske, 1985; Ronfort, 1999), and these provide little consensus on the fundamental question of whether equilibrium values of inbreeding depression should be greater or smaller in tetraploids than diploids.

Of the theoretical work, Lande & Schemske's (1985) conclusion that tetraploids should maintain between half (under complete recessivity) and the same (for partially recessive alleles) inbreeding depression as diploids has received most attention. However, other workers have reached different conclusions (e.g. Bennett, 1976). In particular, Ronfort (1999) has provided a comprehensive treatment of these issues in autotetraploids and has highlighted the complexities involved. As in the diploid case, predictions depend on such factors as the mutation rate to deleterious alleles, their selection coefficients, the degree of dominance between alleles and epistatic interactions among loci (Ronfort, 1999). For polyploids, the increased number of potential dominance interactions between alleles complicates the expectations substantially. In the special case of complete recessivity, equilibrium inbreeding depression in diploids and autotetraploids is expected to be equal. Under partial dominance, however, several of the scenarios investigated by Ronfort (1999) predicted higher inbreeding depression in diploids than autotetraploids, whereas others predicted the reverse. It appears that without knowing the selection and dominance coefficients associated with genotypes carrying alleles at different

dosages (e.g. *AAaa* vs. *AAAA*), it does not seem possible to make very general predictions about equilibrium values of inbreeding depression, and thus about the evolutionarily stable selfing rate (Ronfort, 1999). It is worth noting that the approach taken in all of these studies requires selection-mutation equilibrium, and it seems unlikely that a neo-polyploid will fulfil this criterion. Indeed, depending on the rate of equilibration relative to the process of diploidization, selection-mutation equilibrium may never be met in polyploid lineages with polysomic inheritance.

As far as empirical work is concerned, the few relevant comparisons of inbreeding depression between polyploid lineages and their putative diploid progenitors have yielded mixed results. Early work was based largely on polyploid forage and crop plants (e.g. Busbice & Wilsie, 1966; Dewey, 1966). These studies reported increased levels of inbreeding depression in polyploids relative to their diploid counterparts. However, because product yield (rather than fitness) was measured, and because the recent history of the respective polyploid lineage was not always clear, interpretation of these results is difficult. Of the small number of studies examining inbreeding depression in natural polyploid populations (whether in relation to selfing rates or not), most have not attempted a comparison with their diploid relatives (e.g. Inoue, Masuda & Maki, 1998; Dudash & Fenster, 2001; Galloway *et al.*, 2003). Only Husband & Schemske (1997) and Rosquist (2001) have conducted diploid-polyploid comparisons of inbreeding depression, and both studies found it to be lower in the polyploid lineages. We have been unable to find any empirical studies of inbreeding depression that take into account both variation in the selfing rate and the ploidy level. Again, the most convincing, albeit indirect, evidence for the importance of inbreeding depression as a mediating influence in the interaction between polyploidy and the sexual system is the association between polyploidy and dioecy in lineages derived from SI hermaphrodites (Miller & Venable, 2000; Charlesworth, 2001).

There is clearly a great need for further theoretical and empirical work on inbreeding depression in polyploid-diploid comparisons. Here, it will be important to distinguish between inbreeding depression in neo-polyploids, which will influence the evolution of the sexual system immediately following genome duplication, and inbreeding depression at mutation-selection equilibrium following any possible purging. It would thus be particularly instructive to conduct such comparisons between diploid lineages and established polyploids as well as artificially induced polyploid lineages derived from the same putative progenitors.

As yet, there are no estimates of inbreeding depression for *M. annua* populations, but data from this species would be useful because of the existence of both

inbred (monoecious) and outbred (androdioecious or subdioecious) polyploid populations in addition to the outbred diploids. Certainly, until we have good estimates of inbreeding depression in the various genetic backgrounds of *M. annua*, it will remain difficult to evaluate the importance that this factor may have had in the evolution and continued maintenance of self-fertile monoecy in the polyploid populations of the species.

REPRODUCTIVE ASSURANCE

We have hypothesized that self-fertile monoecy is maintained in *M. annua* through selection for reproductive assurance under demographic conditions in which among-population gene flow is limited and population size fluctuations associated with metapopulation dynamics are more severe (Pannell, 1997a, 2000; Fig. 3). Within the distribution of hexaploid *M. annua* in the Iberian Peninsula, clines between androdioecious and monoecious zones repeatedly coincide with a clear transition in the population structure of the species. In particular, monoecious populations are typically small and geographically isolated from one another, whereas populations in the corresponding androdioecious zone are more common, less geographically isolated and orders of magnitude larger. This trend is also reflected in patterns of both morphological and isozyme diversity, which seem to be higher in androdioecious populations and low in monoecious regions (D. J. Obbard, S. M. Eppley, S. A. Harris & J. R. Pannell, unpubl. data). Higher historic levels of inbreeding in monoecious than in androdioecious regions, estimated indirectly on the basis of patterns in sex allocation (J. R. Pannell, unpubl. data), provide further support for the metapopulation model in *M. annua*.

Although direct estimates of density-dependent selfing rates and rates of population turnover remain outstanding, there are good indications that metapopulation structure and dynamics do differ between monoecious and androdioecious zones along predicted lines. Because these comparisons can be made independently of ploidy differences, they suggest that selection on the sexual system itself is also likely to have contributed to the maintenance of differences in the sexual system across the two diploid–hexaploid transitions. Nonetheless, it is important to bear in mind that other genetic and ecological differences between ploidy levels may have played, and continue to play, a substantial role in shaping the geographical distribution and population structures of the two cytotypes. To what factors, for example, can we attribute the fragmented population structure observed in hexaploid relative to diploid zones on either side of the Catalonian contact?

We have already considered the possible importance of inbreeding depression in allowing the evolution of self-fertile hermaphroditism. This, on its own, may have contributed to the ability of self-fertilizing monoecious colonizers to expand their range into new or inhospitable environments where small population sizes and mate limitation are prevalent. Such a scenario may also account for the central European distribution of the self-fertilizing tetraploid cytotype of the fern *Asplenium* sp., whose more outcrossing diploid progenitors are confined to the more southerly Mediterranean Basin (Vogel *et al.*, 1999). It is plausible that high inbreeding depression in diploid populations of *Asplenium* has prevented the evolution of self-fertilization and thus also the potential for range expansion (Vogel *et al.*, 1999). As noted earlier, however, our understanding of inbreeding depression in polyploids remains poor.

Two other issues impinge upon the contrasting population structures and distributional ranges displayed by *M. annua* diploids and hexaploids. The first concerns possible ecophysiological changes directly associated with polyploidy that may have allowed the species to invade a new environment, irrespective of its sexual system (cf. Ramsey & Schemske, 2002). The second relates to the post-Pleistocene colonization history of the respective cytotypes and the possible ongoing genetic and ecological interactions between them across zones of primary or secondary contact (Durand, 1963).

ECOPHYSIOLOGICAL NICHE DIFFERENTIATION

It has long been thought that polyploidization may have allowed species to expand their range into novel environments as a result of derived ecophysiological differences relative to diploid progenitors (reviewed in Levin, 2002; Ramsey & Schemske, 2002). Again, it is important here to distinguish between neopolyploids, in which polyploidization directly causes physiological alterations, and long-established polyploids, in which changes may be the result of subsequent adaptive evolution within the new environment that was occupied for other reasons (Ramsey & Schemske, 2002).

The increased DNA content brought about by polyploidization may lead directly to increased cell size, reduced stomatal density and slowed cell division (see Bennett, 1987). These changes may then influence ecophysiological traits such as transpiration and gas exchange (summarized in Levin, 2002), and life-history traits such as generation time (Bennett, 1987). Increased gene dosage and disruption of the control of gene expression may cause changes in the level and pattern of gene expression (Guo, Davis & Birchler, 1996; Comai *et al.*, 2000; Wendel, 2000; Osborn *et al.*, 2003). It has also been widely argued that increased

levels of heterozygosity may be an important source of increased vigour, ecological tolerance and thus colonizing ability (Bingham, 1980; Stebbins, 1980, 1985; Tomekpe & Lumaret, 1991; Brochmann & Elven, 1992). In the case of allopolyploids, genome duplication has been claimed to represent 'a permanent combination of adaptive strategies and ecophysiological capabilities inherited from different diploid progenitor species' (Brochmann & Elven, 1992), which thus allows species range expansions (see also Ainouche, Baumel & Salmon, 2004; Soltis *et al.*, 2004 – both this issue). It should be noted that there is little theoretical or empirical evidence to support the notion that increased heterozygosity and gene duplication leads to such increases in fitness.

In *M. annua*, the transition from diploidy to hexaploidy in Catalonia occurs across a gradient of increasing aridity, pointing to possible ecophysiological differences between the cytotypes in this region. However, preliminary results from reciprocal transplant experiments have so far provided no evidence for the hypothesis of ecophysiological local adaptation across the zone of contact between diploid dioecious and hexaploid monoecious population (R. J. A. Buggs & J. R. Pannell, unpubl. data). More generally, gradients in increasing aridity on the Iberian Peninsula and in Morocco repeatedly correspond to transitions from dioecy or androdioecy to monoecy (i.e. from separate to combined sexes). It thus seems possible that transitions in ploidy correlate with shifts in breeding system as a result of selection for reproductive assurance in metapopulations with increasing isolation among demes and higher extinction rates (Pannell, 1997a). This would explain observations of increased reproductive failure of transplanted females due to mate limitation in the region occupied by monoecious populations in Catalonia (R. J. A. Buggs & J. R. Pannell, unpubl. data).

INTERACTION ACROSS ZONES OF CONTACT

The expansion of species ranges generally is thought to be limited by a balance between natural selection, bringing about local adaptation at the range boundary, and gene flow from more central parts of a species range, which impedes local adaptation (Kirkpatrick & Barton, 1997; Lenormand, 2002). The formation of barriers to gene flow between populations may therefore remove this constraint on local adaptation, allowing gene frequencies to change in response to local selection. Polyploidization may represent an important source of such a barrier if it causes reduced gene flow between diploids at the centre of the range and new polyploid lineages at its extremity.

The form of genetic isolation between diploids and their polyploid derivatives required by this hypothesis

may be either post-zygotic or pre-zygotic, the latter of which may be due to changes in the sexual system. Table 1 lists several examples of pre-zygotic isolation due to changes in the sexual system that have been observed between natural diploid and polyploid populations, including differences in flowering time, pollinator sorting and shifts from dioecy to monoecy or from sexual reproduction to apomixis. In some cases, these changes in the sexual system may be a direct and immediate result of polyploidization *per se*, which may contribute to the ability of polyploids to establish under a reduced influence of minority cytotype exclusion (Levin, 1975; Felber, 1991; Husband, 2000; see below). In other cases, such mechanisms may have evolved gradually as a result of reinforcement selection to avoid the negative consequences of interploidy fertilizations. Although the conditions under which reinforcement may occur are limited (Barton & Hewitt, 1985), in the context of polyploidy it seems most likely to be found in secondary contacts that have a mosaic of patches of differing cytotypes, exposing a high proportion of individuals to selection (Cain, Andreassen & Howard, 1999).

In the examples listed in Table 1 (see also Petit, Betagnolle & Felber, 1999), it is not always possible to distinguish between isolating mechanisms as a direct result of polyploidy and those that have arisen in response to reinforcement. In the case of triploid *Taraxacum*, the chromosome number is directly responsible for the shift to apomixis (see Table 1 for references). Conversely, in the case of *Dactylis glomerata*, selection seems to have acted subsequent to polyploidization because flowering-time divergence has been found in natural sympatric diploid–tetraploid populations (Lumaret *et al.*, 1987; Lumaret & Barrientos, 1990), but not between diploid and neo-tetraploid lineages (Bretagnolle & Lumaret, 1995).

Post-zygotic isolation as a result of low seed viability appears to be particularly common in diploid–polyploid contact zones. In *Arabidopsis thaliana*, this reduction in seed viability has been attributed to disrupted endosperm development, caused by an imbalance in the ratio of maternal and paternal genomes (Scott *et al.*, 1998). Inter-ploidy hybrids may also display reduced fertility, e.g. due to the high incidence of unpaired chromosomes at meiosis (Sybenga, 1975). In mixed-ploidy populations, both of these processes are expected to give rise to frequency-dependent selection, with a fitness disadvantage to the minority cytotype (Levin, 1975; Felber, 1991; Rodriguez, 1996a, b; Husband, 2000). Minority cytotype exclusion places a constraint on the establishment of new polyploids (but see Husband, 2004 – this issue), and it may also give rise to hybrid 'tension-zone' dynamics at secondary contacts between populations with different ploidy levels.

Tension zones are boundaries of contact between

Table 1. Pre-zygotic reproductive isolating mechanisms involving the sexual system found in wild diploid–polyploid contacts

| Species | Ploidy levels | Pre-zygotic breeding system isolation mechanism | Inter-ploidy hybrid occurrence | References |
|------------------------------------|---------------|---|--------------------------------|---|
| <i>Anthoxanthum alpinum</i> | 2x,4x | Flowering time difference | Rare | (FelberGirard, Felber & Buttler, 1996) |
| <i>Mercurialis annua</i> | 2x,6x | Dioecy/monoecy? | Common | (Durand, 1963) |
| <i>Lotus alpinus/ corniculatus</i> | 2x,4x | Flowering time difference | Never found | (Gauthier, Lumaret & Bedecarrats, 1999) |
| <i>Plantago media</i> | 2x,4x | Flowering time difference | Very rare | (Van Dijk <i>et al.</i> , 1992; Van Dijk & Bijlsma, 1994; Van Dijk & Bakx-Schotman, 1997) |
| <i>Chamerion angustifolium</i> | 2x,3x,4x | Flowering time difference; pollinator sorting | 7% | (Husband & Schemske, 1998; Husband, 2004) |
| <i>Arrhenatherum elatius</i> | 2x,4x | Flowering time difference; high selfing rate? | 1% | (Petit <i>et al.</i> , 1997; Petit & Thompson, 1997) |
| <i>Heuchera grossulariifolia</i> | 2x,3x,4x | Pollinator sorting | 1.4% | (Segraves & Thompson, 1999; Segraves <i>et al.</i> , 1999) |
| <i>Carya ovata/tormentosa</i> | 2x,4x | High selfing rate? | ? | (McCarthy & Quinn <i>et al.</i> , 1990) |
| <i>Taraxacum</i> spp. | 2x,3x | Sexual/apomictic | Common | (Menken, Smit & DenNijs, 1995) |
| <i>Claytonia virginica</i> | 2x,4x | Flowering time difference | Common | (Lewis, 1976) |
| <i>Dactylis glomerata</i> | 2x,4x | Flowering time difference | No adult found | (Lumaret <i>et al.</i> , 1987; Lumaret & Barrientos, 1990; Bretagnolle & Lumaret, 1995) |

potentially interbreeding populations whose hybrid progeny suffer reduced fitness (Barton & Gale, 1993; Kruuk *et al.*, 1999). A particularly important implication of their dynamics is that the relative distribution of the two populations in contact depends largely on the (density-dependent) rates and direction of gene flow between them, and notably not on ecological differentiation or adaptation to different ecological environments (Barton & Hewitt, 1985). Contact zones between diploid and tetraploid *Plantago media* in the Pyrenees (Van Dijk, Hartog & Vandelden, 1992; Van Dijk & Bakx-Schotman, 1997), and *Centaurea jacea* in north-east Belgium (Hardy *et al.*, 2000, 2001) appear to be good examples of tension zones. By contrast, in diploid and polyploid populations of *Dactylis glomerata* (Lumaret *et al.*, 1987; Lumaret & Barrientos, 1990) and *Claytonia virginica* (Lewis, 1976) tension-zone dynamics are mitigated by reproductive isolation due to flowering phenology. Similarly, sympatry is possible for different ploidy levels of *Heuchera grossulariifolia*, partly as a result of differences in flower phenology and pollinator sorting (Segraves & Thompson, 1999).

The position of tension zones is sensitive to differential rates of gene flow from one cytotype to the other, or to 'anisotropy'. This results in the displacement of the cytotype with poorer dispersal by the better disperser (Barton & Hewitt, 1985). Anisotropy can be due

in particular to differences in phenology, the mating system or sex allocation. The best evidence for asymmetry in pre-zygotic isolation between related cytotypes is provided by populations in a zone of sympatry between diploid and tetraploid *Chamerion angustifolium*, where differences in flowering time, pollinator fidelity and pollen competition mean that pollen flow from tetraploids to diploids is much more common than vice versa (Husband & Schemske, 2000; Husband, 2000; Husband *et al.*, 2002). Diploid fitness is thus reduced compared with tetraploids in the contact zone. This may ultimately allow tetraploids to expand their range, although the dynamics in this tension zone appear to be dampened by ecological sorting, perenniality, clonal reproduction and variable triploid fitness (Husband *et al.*, 2002; Husband, 2004).

The effect of interploidy differences in sex allocation almost certainly play a role in shaping the contact zones between diploid and hexaploid populations of *M. annua* in north-eastern and north-western Spain. Because of the much greater pollen productivity of males relative to monoecious plants (Pannell, 1997b), pollen flow out of dioecious populations will be substantially higher than out of monoecious ones. Moreover, the fact that males disperse their pollen from erect pedunculate inflorescences, in contrast to the staminate flowers of monoecious individuals that are

subsessile in the leaf axils, will no doubt contribute to the anisotropy due to sex allocation (Pannell, 1997c). The degree of anisotropy in *M. annua* contact zones and the extent to which self-fertilization in polyploid populations may mitigate gene flow from diploid individuals are areas of active research in our laboratory. Certainly, low levels of gene flow among monoecious populations are suggested by higher levels of population differentiation compared with dioecious populations (D. J. Obbard, S. M. Eppley, S. A. Harris & J. R. Pannell, unpubl. data).

The effects on fitness of interploidy hybridization in *M. annua* appear to be severe. Diploid and hexaploid *M. annua* do cross-pollinate in several natural sympatric populations in Catalonia, but the resulting tetraploid progeny are sterile (Durand, 1963; R. J. A. Buggs & J. R. Pannell, unpubl. data). The relative rarity of mixed-ploidy populations in Catalonia and Galicia, as well as the very narrow zone within which populations of either cytotype are found, supports a hypothesis of strong tension-zone dynamics in *M. annua*. Moreover, the contact zone in Catalonia occurs in an area of locally low rainfall along a relatively narrow coastal strip where *M. annua* populations are rare, a fact that is consistent with tension-zone models that predict maximum steepness in clines to occur in areas of low population density (Barton & Hewitt, 1985).

CONCLUSIONS

Despite the clear importance that the sexual system must have in regulating the success of polyploid lineages, and the manifold effects that polyploidy is likely to have on the sexual system in turn, there is remarkably little firm theoretical or empirical research to substantiate the interactions we have discussed in this article. It is safe to conclude that further work aimed at investigating each of the proposed causal links outlined in Figure 1 would be valuable. The poor theoretical understanding and the limited direct empirical support for the relationship between polyploidy and inbreeding depression, both in neopolyploids and during the course of subsequent genome evolution, are particularly striking and worthy of redress. This is not only because of the general importance of understanding the maintenance of genetic load in populations of plants and animals, but also because of the fundamental role that inbreeding depression is believed to have in regulating the stability of the mating system. The association between polyploidy and dioecy, and its proposed explanation (Miller & Venable, 2000), show convincingly that inbreeding depression is involved in an important way in the interactions between polyploidy and the sexual system (Charlesworth, 2001).

The association between ploidy and the sexual sys-

tem in *M. annua* is of course quite the reverse to that exposed more generally by Miller & Venable (2000). Rather than viewing this as an annoying exception, we suggest that it reveals more tellingly the potential importance that selection for reproductive assurance has had in continuing to maintain monoecy in this annual colonizer of ephemeral habitat. The relationship between combined vs. separate sexes and population structure, which we have described for hexaploid *M. annua* independent of ploidy differences, adds weight to our hypothesis. Nevertheless, our studies of *M. annua* to date have thrown up more questions than answers, and we are still far from understanding the evolutionary significance of the striking variation in ploidy and sexual systems we observe.

We are currently conducting a detailed examination of the ecological and genetic interactions that occur across transitions of ploidy and sexual systems in the species, including the use of transplant experiments to assess the relative importance of reproductive assurance, ecophysiology and tension-zone dynamics in determining where these transitions occur. We are also analysing patterns of genetic polymorphism in zones represented by contrasting cytotypes and sexual systems to test predictions regarding the demographic history and structure of the respective populations. Although still incomplete, data on patterns of isozyme variation in populations throughout Europe and the Mediterranean Basin strongly implicate Pleistocene refugia in the eastern Mediterranean for diploid *M. annua* and in southern Iberia or North Africa for tetraploids and hexaploids (D. J. Obbard, S. M. Eppley, S. A. Harris & J. R. Pannell, unpubl. data). This should caution us to consider the hypotheses we have discussed in light of the phylogeographic history of the *M. annua* species complex. This, of course, is likely to be idiosyncratic in its details, but continued study of *M. annua* is nevertheless likely to fill important gaps in our general understanding of polyploidy and the sexual system in plants.

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