Providence College

From the SelectedWorks of Maia F. Bailey

January 2007

A field guide to models of sex-ratio evolution in gynodioecious species

Contact Author

Start Your Own SelectedWorks

Notify Me of New Work

Available at: http://works.bepress.com/maia_bailey/1

Forum

Oikos 000: 000–000, 2007
doi: 10.1111/j.2007.0030-1299.15315.x,
Copyright © Oikos 2007, ISSN 0030-1299
Subject Editor: Lonnie Aarssen, Accepted 11 June 2007

A field guide to models of sex-ratio evolution in gynodioecious species

Maia F. Bailey and Lynda F. Delph

M. F. Bailey (mabailey@indiana.edu) and L. F. Delph, Dept of Biology, Indiana Univ., Jordan Hall, 1001 East Third Street, Bloomington, IN 47405-3700, USA.

Gynodioecious plant species, species in which individuals are females or hermaphrodites, are ideal systems for studying connections between genetics, ecology, and long-term evolutionary changes because sex determination can be complex, involving cytoplasmic and/or nuclear genes, and sex ratio is often variable across landscapes. Field data are needed to evaluate the many theories concerning this breeding system. In order to facilitate the gathering of relevant data, this paper introduces the four types of gynodiocy (nuclear, nuclear-cytoplasmic and stochastic gynodioecy plus subdioecy), describes example species and expected patterns, discusses the various forces that drive the evolution of female frequencies, and gives concrete advice on where to start collecting data for different systems. For species in which females are relatively rare, we recommend reciprocal crosses to determine if sex-determination is nuclear or nuclear-cytoplasmic along with a search for correlations between female frequencies and ecological factors. For species in which females are common and sex ratios are highly variable, we recommend looking at female offspring sex ratios to determine if females are primarily produced in ephemeral epidemics.

In the course of this discussion, we argue that the majority of natural gynodioecious species will have complex sex determination in which multiple cytoplasmic male sterility (CMS) genes interact with multiple nuclear restorers of fertility. Sex-ratio evolution in such species will be primarily influenced by fitness differences among hermaphrodites (costs of restoration) and less influenced by fitness differences between the sexes (compensation). Metapopulation dynamics alone may explain population sex ratios of species in which females are associated with marginal environments or hybrid zones; however, we feel that in most cases equilibrium forces within populations and metapopulation dynamics among populations each explain portions of the sex-ratio pattern.

UNCORRECTED PROOF

Dimorphic plant breeding systems are excellent systems for studying the interplay of genetic architecture and ecology in evolution. The fate of a novel sex-determining gene depends both on its placement within the genome – e.g. on a somatic chromosome linked to other genes, on a sex chromosome, on a plasmid, or on an organellar genome – and its ecological context. Dimorphic breeding systems have been shown to have several ecological correlates (reviewed by Sakai and Weller 1999) suggesting that certain ecological contexts select for sexual specialization. For example unisexual plants are often associated with extreme physical environments. Some dimorphic breeding systems are especially variable among populations prompting researchers to ask whether sex ratio as well as presence or absence of unisexuals reflects differences in ecology. A wealth of models exists describing the invasion of unisexuals and sex-ratio evolution. These models can be used to evaluate theories connecting sex ratios to local ecology.

Gynodioecy is a breeding system of plants in which populations consist of hermaphrodite and female individuals (see Glossary). It is both a common and widespread polymorphism describing approximately 7% of all flowering plants and found in many angiosperm families (Richards 1986). Gynodioecy has been functionally identified in many species of ecological and economic interest, but relatively few of these species have had their breeding system carefully examined. This review is intended to be a useful guide

Box 1. A key to the different kinds of gynodioecious breeding systems

1. Reciprocal crosses among hermaphrodites yield offspring with equivalent sex ratios. Frequencies of females are fairly consistent among populations and are less than or equal to 50%. 2
1. Reciprocal crosses among hermaphrodites yield offspring with different sex ratios. Frequencies of females vary among populations and may reach above 50%. 3
    2. Hermaphrodites rarely produce offspring through seeds. Some true males may be present. *subdioecy*
    2. Hermaphrodites regularly produce significant numbers of offspring as a seed parent, although considerably fewer than females. ..... *nuclear gynodioecy*
3. Females are rare and only found within a few, often ecologically grouped, populations. *stochastic gynodioecy*
3. Female sex ratios are often above 50% and vary widely among populations. .... *nuclear-cytoplasmic gynodioecy*

for field biologists who are interested in gynodioecy and the maintenance of females in gynodioecious populations and who want to evaluate current models of sex-ratio evolution in gynodioecious populations with respect to their observations in the field. There are several steps that are required to go from a general observation of functional gynodioecy to the kind of detailed assessment needed to effectively choose a candidate model to describe sex-ratio evolution in a particular system. To facilitate this matching of natural populations to models, we have divided up gynodioecy into four types (nuclear, nuclear-cytoplasmic, and stochastic gynodioecy plus subdioecy) and devised a key to determine which type of gynodioecy best describes a particular species (Box 1).

Using our key

To accompany our key, we have included a glossary, a description of each type of gynodioecy including example species, a table suggesting appropriate models of sex-ratio evolution for each type of gynodioecy (Table 1) and a graphical comparison of the two main equilibrium models (Fig. 1).

Dichotomy 1

The first dichotomy concerns the genetic basis of sex determination. Because nuclear and cytoplasmic genes have very different patterns of inheritance (primarily biparental and maternal, respectively), the presence or absence of cytoplasmic sex-determining genes has a major effect on how sex ratios evolve. You can determine if there are cytoplasmic sex-determination genes in your species of interest by comparing offspring sex ratios from reciprocal crosses. In these types of crosses, pairs of hermaphrodites are used to produce two sets of offspring. To make the first set of offspring, one hermaphrodite is the seed parent and the other is the pollen donor; to make the second set, their roles are reversed. The resulting two sets of offspring will have the same distribution of nuclear genes but can differ for cytoplasmic genes that are primarily maternally inherited. In order to maximize the likelihood of choosing two hermaphrodites with different cytoplasmic sex-determining genes, it is recommended that several hermaphrodites should be used to make multiple reciprocal crosses and that a portion of those crosses include hermaphrodites from different populations (Kaul 1988).

Table 1. The four major types of gynodioecy, their models and example species. In the equations, p is the frequency of females, x is compensation (the excess seed fitness of females as compared to hermaphrodites), and y is cost of restoration (the loss of fitness in hermaphrodites with excess restorers as compared to hermaphrodites without excess restorers).

Type of gynodioecy	Models	Example species (observed female frequencies)
Nuclear	$p=(x-1)/2x$ (Lewis 1941)	*Cucurbita foetidissima* (0.21–0.65)[1]
	See also:	*Fragaria virginiana* (0.20–0.44)[2]
	Lloyd 1974, 1975	*Fuchsia excorticata* (0.04–0.40)[3]
	Charlesworth 1981	*Phacelia linearis* (0–0.16)[4]
Subdioecy	Maurice et al. 1993	*Hebe subalpina* (0.5)[5]
	Schultz 1994	*Ochradenus baccatus* (0.5)[6]
	Delph and Wolf 2005	
Stochastic	Frank 1989	*Nemophila menziesii* (0–0.53)[7]
	McCauley and Taylor 1997	*Raphanus sativus* (0–0.21)[8]
	Pannell 1997	*Thymus vulgaris* (0.05–0.95)[9]
	Couvet et al. 1998	
Nuclear-cytoplasmic	$p>0$ if $x+2y>1$	*Silene vulgaris* (0–0.75)[10]
	(Gouyon et al. 1991)	
	See also: Bailey et al. 2003	

[1] Kohn 1989; [2] Ashman 1999; [3] Godley 1955; [4] Eckhart 1992; [5] Delph 1990b; [6] Wolfe and Shmida 1997; [7] Barr 2004; [8] Murayama et al. 2004; [9] Dommée et al. 1978; [10] McCauley et al. 2000.

UNCORRECTED PROOF

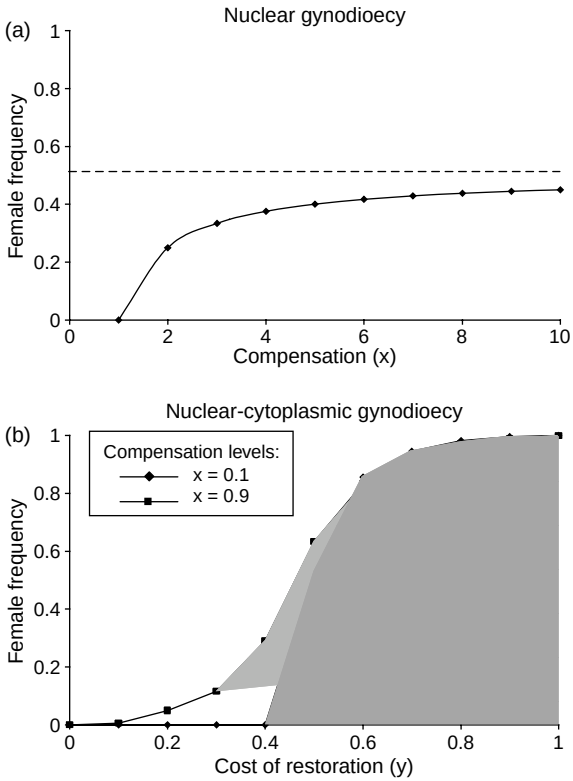

Fig. 1. Female frequency predictions of two equilibrium models. Sex ratio in (a) nuclear gynodioecy is determined by between-sex fitness differences (compensation) alone while (b) both fitness differences among hermaphrodite genotypes (cost of restoration) and between-sex fitness differences (compensation) affect sex ratio in nuclear-cytoplasmic gynodioecy. Cost of restoration has a greater affect on predicted sex ratio than compensation as can be seen in the gross similarity of predictions under low (x = 0.1) and high (x = 0.9) compensation. Gray areas represent the range of predicted female frequencies under dynamic equilibria.

Dichotomy 2

If the sex ratios of the two sets of offspring do not differ statistically, your species is probably nuclear gynodioecious or subdioecious. It is important to note that species that are currently nuclear gynodioecious can have evolved from populations with cytoplasmic sex-determining genes but no longer have genetic variation for these genes (Fishman and Willis 2006). Furthermore, while subdioecy can evolve from gynodioecy types that include cytoplasmic sex-determining genes, theory predicts that nuclear genetic variation will primarily determine sex ratios as hermaphrodites reduce their investment in female function and populations evolve toward dioecy (Maurice et al. 1993). Therefore, gynodioecy (both nuclear and nuclear-cytoplasmic) and subdioecy form a continuum in which hermaphrodites make significant numbers of offspring via seeds in gynodioecious species and rarely make offspring via seeds in subdioecious species.

Dichotomy 3

If the offspring sex ratios differ, sex determination in your species is nuclear-cytoplasmic. Although sex determination in some populations has been shown to be primarily cytoplasmic (e.g, in one population of *Beta vulgaris*; LaPorte et al. 2001), no known species have strict cytoplasmic sex determination (Charlesworth 1981). Once a cytoplasmic component to sex determination is established, the next dichotomy is to determine if equilibrium forces at the level of the population or metapopulation dynamics are primarily responsible for the presence of females in populations. Although in most cases we will argue that both equilibrium forces and metapopulation dynamics act together to determine sex ratio, there are a few instances in which it appears that females would not be present at a local equilibrium but are produced by stochastic, metapopulation forces such as gene flow among populations. Hybridization between diverging populations can reveal latent gynodioecy by uncovering CMS alleles that are completely restored in each parental population. For example, the presence of females in *Nemophila menziesii* is associated with hybridization between populations that have diverged for corolla color (Barr 2004). We describe the breeding system of such species as 'stochastic' gynodioecy.

Although genetic drift probably limits the number of cytoplasmic sex-determining genes found in gynodioecious populations, there is less evidence that genetic drift reduces variation of nuclear sex-determining genes within those populations (Olson and McCauley 2002, Murayama et al. 2004). Therefore, if females are not restricted to a few populations, models based on metapopulation dynamics alone cannot explain why selection has not acted to increase frequencies of nuclear restorers-of-male-fertility alleles and thereby remove females from populations. In these cases, equilibrium models suggest that selection among hermaphrodites (i.e. a cost of restoration – see Glossary for a technical definition) may be acting to reduce frequencies of nuclear restorers-of-fertility alleles and thus maintain females in older populations (Delannay et al. 1981, Gouyon et al. 1991, Bailey et al. 2003). When there is local genetic polymorphism for both cytoplasmic and nuclear sex-determining genes, we describe the breeding system as nuclear-cytoplasmic.

In most gynodioecious populations, sex ratio is probably a function of both gene flow among populations and selection within populations; therefore, stochastic and equilibrium gynodioecy (both nuclear and nuclear-cytoplasmic) are a continuum in which

UNCORRECTED PROOF

metapopulation dynamics have greater or lesser effects, respectively, on female frequencies.

Nuclear gynodioecy

Models, ecology and example species

Models of nuclear gynodioecy emphasize differences among females and hermaphrodites that can be affected by ecological context. Several models of sex-ratio evolution describe nuclear gynodioecy (Table 1); however, most can be considered as refinements on Lewis (1941). Lewis' basic premise is that sex ratio is controlled by differences in seed fitness between females and hermaphrodites. Females will be maintained if they make as many successful gametes as hermaphrodites ($x > 1$; Fig. 1a). The difference in seed fertility between females and hermaphrodites can be influenced by ecology and sexual dimorphism, e.g. inbreeding depression (Lloyd 1975, Charlesworth and Charlesworth 1978, Charlesworth 1999), pollination efficiency (Lloyd 1974), plasticity of fruit production (reviewed by Delph 2003), natural enemies (Mutikainen and Delph 1996, Marshall and Ganders 2001, Ashman 2002, Collin et al. 2002), physiology (Delph 1993, 1999, Caruso et al. 2003), timing of allocation to reproduction (Delph 1999) and vegetative reproduction (Stevens and Van Damme 1988). When ecological interactions vary between populations, equilibrium sex ratios may vary; however, compared to stochastic and nuclear-cytoplasmic gynodioecy, female frequencies are expected to be fairly stable among populations and below 50% in general. Four species have been shown to be gynodioecious as a consequence of nuclear sex-determination: *Cucurbita foetidissima* (Kohn 1989), *Fragaria virginiana* (Ahmadi and Bringhurst 1991), *Fuchsia excorticata* (Godley 1955), and *Phacelia linearis* (Eckhart 1992).

Subdioecy

Models, plasticity and example species

Subdioecy evolves when females are stably present over long periods of time allowing hermaphrodites to maximize their fitness by shifting resources away from female function and toward male function (Charlesworth and Charlesworth 1978, Charlesworth and Guttman 1999). Models describe when strict males can invade such a system of 'fruiting' males and females completing the evolution of dioecy from gynodioecy (Lloyd 1976, Charlesworth and Charlesworth 1978, Maurice et al. 1993, Schultz 1994). As hermaphrodites are displaced by true males, female frequencies should approach 50% (Lloyd 1976). This final step can be complicated when fruit production is plastic such that hermaphrodites may not produce fruit at a canalized rate, but rather produce fruit when conditions are favorable (Delph and Wolf 2005). Examples of subdioecious species are *Hebe subalpina* (Delph 1990b) and *Ochradenus baccatus* (Wolfe and Shmida 1997).

Stochastic gynodioecy

Epidemics of females

In stochastic gynodioecy, female epidemics sweep through populations due to founder effects or gene flow. Because cytoplasmic male-sterility (CMS) genes are primarily inherited through seeds while both pollen and seeds can spread nuclear restorers of male fertility (restorers), the distribution of these two types of genes might differ because of differences in the ecology of seed and pollen distribution. In species with stochastic gynodioecy, females result from the periodic invasion of populations by CMS genes ahead of their corresponding nuclear restorers. When this happens, any seed-fitness advantage of the females will cause the spread of the cytoplasmic trait. Unlike for a nuclear-feminizing gene, CMS genes do not need to confer a two-fold seed-fitness advantage in order to equal the gamete output of hermaphrodites because cytoplasmic genes are primarily maternally inherited (Lewis 1941). In other words, when it comes to cytoplasmic fitness, pollen grains count for nothing, so losing the ability to make pollen does not affect the fitness of CMS genes (but can have a negative affect on the nuclear genes in the same individual). Therefore, any seed-fitness advantage associated with being female, such as reallocation of resources from staminate tissues (Darwin 1877), will cause CMS frequencies to increase every generation causing an epidemic of females until a nuclear restorer gene either evolves or migrates into a population (Frank 1989).

Models of stochastic gynodioecy

Models describing stochastic gynodioecy focus on the differential effects of metapopulation forces such as founder effects, migration rates and local extinction probabilities on frequencies of CMS and nuclear restorer genes within and among populations (Table 1). A fitness difference between females and hermaphrodites (compensation) is often included as a parameter, but a fitness difference among hermaphrodites (cost of restoration) is usually not. In the one model (Frank 1989) that includes a fitness difference among hermaphrodites, the assumed fitness effects are insufficient to maintain females under equilibrium conditions within populations. In general, these models make a

UNCORRECTED PROOF

connection between female frequency and population-level fitness depending on whether they increase population fecundity or cause pollen-limitation, respectively. Selection among populations then controls the evolution of sex ratio. Note that these models can be deterministic in their construction (McCauley and Taylor 1997, Couvet et al. 1998) but are called 'stochastic' because they predict spatial and temporal stochasticity of female frequencies.

Expected patterns and example species

Species with pure stochastic gynodioecy exist, however, in most species both stochastic and equilibrium forces explain the distribution of females. In species with stochastic gynodioecy, high frequencies of females are associated with young colonies and/or populations with high gene flow experiencing epidemics of females. Once nuclear restorers are present within a population, selection patterns change driving the population towards equilibrium such that females are lost or are considerably less common. In *Nemophila menziesii* (Barr 2004), significant numbers of females are found only in hybrid zones between populations that differ for corolla color. Occasionally, some species experience frequent epidemics of females but also maintain females at much lower frequencies under equilibrium conditions. For example in *Thymus vulgaris*, female frequencies vary widely among populations (5–95% females; Dommée et al. 1978) and average 63% overall (Manicacci et al. 1998). The overall high frequency of females is driven by founder effects causing ephemeral cytoplasmic sex determination (Oliveri et al. 1990, Couvet et al. 1998). In contrast, the ubiquitous distribution of females can be explained by the stabilizing effect of high compensation (x, see Glossary) in this species ($0.5 \leq x \leq 2.5$ in *T. vulgaris*; Couvet et al. 1986). In other words, as nuclear restorers migrate into populations they are prevented from spreading to fixation by high differences in seed fitness between females and hermaphrodites. Indeed, in a survey of six populations no true-breeding females were found, indicating that sex determination in those populations was locally nuclear-cytoplasmic (Manicacci et al. 1998). *T. vulgaris* is a good example of a species in which both stochastic and equilibrium models can be of use in describing patterns of sex-ratio variation and evolution.

Nuclear-cytoplasmic gynodioecy

Model predictions

Models describing this type of gynodioecy use among-sex differences like previous models but also introduce a new parameter. Specifically, they predict that both among-sex fecundity differences (compensation) and differences in fitness among hermaphrodite genotypes (cost of restoration) affect equilibrium female frequencies (Fig. 1b). Compensation can be any difference in fitness between hermaphrodites and females and can be associated with any sexually dimorphic traits such as allocation to seeds (Darwin 1877), potential for inbreeding (Lloyd 1975), male-biased herbivory (Ashman 2002), etc. (Nuclear gynodioecy above). Cost of restoration is a difference in fitness among classes of hermaphrodites based on their restorer genotype. Specifically, cost of restoration is a negative pleiotropic fitness effect of restorers. Theoretical predictions (Delph et al. 2007) and limited data (Bailey 2002) suggest that cost of restoration will primarily affect pollen fitness rather than seed fitness.

These models differ from previous models of gynodioecy in their insensitivity to among-sex differences and in the predicted patterns of female frequencies. For example, equilibrium models indicate that cost of restoration (y, see Glossary) has a greater effect than compensation (x) on the maintenance of females in nuclear-cytoplasmic gynodioecious species – equilibrium female frequencies are predicted to be non-zero if $x+2y>1$ (Gouyon et al. 1991; Fig. 1). Another insight from these models is that dynamic equilibria are also possible in which female frequencies cycle indefinitely with stable minima and maxima. These female-frequency cycles occur when cost of restoration is moderate ($y>0.30$), but will include periods of very low frequencies or absence of females when compensation is small (e.g. when $y=0.4$, female frequency minima are non-zero only when $x \geq 0.5$; Bailey et al. 2003). Therefore, even under conditions that allow for evolution to a local equilibrium (e.g. large, stable populations), populations may vary for sex ratio as a dynamic equilibrium. This prediction of both variation in sex ratio and presence or absence of females complicates discerning between stochastic and nuclear-cytoplasmic evolution; however, populations with females will resemble each other ecologically in stochastic gynodioecy (i.e. females will be associated with young colonies or hybrid zones) but will be independent of ecology in simple nuclear-cytoplasmic gynodioecy.

Ecology and cost

Ecological effects on compensation have been discussed above (see Nuclear gynodioecy). It is possible that local ecology can also effect the expression of cost of restoration via effects on the relationship between investment in male function and realized male fitness. Differences in pollination ecology such as species composition in animal-pollinated plants could magnify

UNCORRECTED PROOF

or reduce fitness differences among classes of hermaphrodites that produce different amounts of viable pollen affecting the magnitude of the cost of restoration.

Example species

Theoretically we expect that the majority of gynodioecious species have nuclear-cytoplasmic sex determination (Charlesworth 1981, Couvet et al. 1990). A quick survey of the literature shows that crossing data in over 20 species is consistent with this type of gynodioecy (Bailey unpubl.). Because very little data exists on cost of restoration, we cannot directly compare model predictions to existing sex ratios for any species and pick an exemplar species. What we can do is indirectly demonstrate the importance of equilibrium forces by discussing an example in which other explanations are insufficient.

Silene vulgaris has been proposed as an example of a gynodioecious species strongly affected by metapopulation forces (Pannell and Dorken 2006); however, stochastic models alone cannot explain the high frequencies (28.2% female on average across populations; McCauley et al. 2000) and prevalence of females (75% of populations are gynodioecious; McCauley et al. 2000). Because colonization does not favor females and migration is more likely to spread restorers than CMS genes, stochastic models can only explain why females might be more rare than we would expect from equilibrium models, not more common. Specifically, isolated females produce far fewer seeds than isolated hermaphrodites in this species (Taylor et al. 1999) suggesting that hermaphrodites will make better colonizers. If colonization cannot explain female epidemics, what about migration? A female migrant should have an advantage because in established populations females have higher seed fitness than hermaphrodites (Taylor et al. 1999) especially when rare (McCauley and Brock 1998); however this advantage is only sufficient to explain the spread of females when sex determination is cytoplasmic (i.e. $x < 1$). Although a few cases have been found of CMS alleles that have temporarily escaped their restorers, females who do not produce at least some hermaphrodite offspring are rare (McCauley et al. 2000) and sex determination is largely nuclear-cytoplasmic within populations (Taylor et al. 2001). Furthermore, in comparing sex ratios of offspring from within- and among-population crosses, migrant offspring were less likely to be female than local offspring (Bailey and McCauley 2005). Because neither colonization nor migration appears to cause female epidemics, we are left with the possibility that the key to female persistence in this species is a cost of restoration.

Where to Start

Scenario 1 – within your species of interest, females are only known from a few populations while most populations contain only hermaphrodites

From our discussion above, you may suspect that you are looking at a case of stochastic gynodioecy. How can you demonstrate the local effects of ecology on sex ratio in this species? We suggest the following first steps:

1. By comparing sex ratios of reciprocally crossed hermaphrodites, determine if sex determination is nuclear or nuclear cytoplasmic. Try to use pairs of hermaphrodites from the same and different populations. If the offspring sex ratios are equivalent, sex determination is nuclear and fitness differences between the sexes and sexual dimorphism are especially important in determining local sex ratio. If the offspring sex ratios are significantly different, sex determination is nuclear-cytoplasmic and founder effects and the ecology of seed and pollen dispersal will drive among-population patterns of female frequencies. Depending on the life history of your species, getting offspring sex ratios may take time, so start your crosses right away. As a bonus, this data may also give indications of population-level pollen limitation or inbreeding depression.
2. Ask yourself – what makes this population special? Is the local ecology different in some way from other populations in which females are absent? This may be a matter of successional age (*Thymus vulgaris* females invade after a fire; Dommée et al. 1978), abiotic resources (*Hebe strictissima* females are more common in dry sites; Delph 1990a), or biotic community (male-biased herbivory may favor females; Marshall and Ganders 2001, Ashman 2002).

Scenario 2 – females are common, often comprising more than 50% of a population, but their frequencies are highly variable among populations

We have argued earlier that models of both stochastic and nuclear-cytoplasmic gynodioecy are useful in interpreting sex-ratio patterns in these kinds of species. With such a wide array of possible forces acting on local sex ratio, what are the best first steps? We suggest you start with these:

1. By looking at the sex ratio of offspring from open-pollinated females, establish whether the restorers

UNCORRECTED PROOF

for the population's CMS types are present within the population. If females make all female offspring, the corresponding restorers are not present, local populations are not in equilibrium and models of stochastic gynodioecy apply. If females produce both female and hermaphrodite offspring, corresponding restorers are present and equilibrium models could be more helpful. (N.B. Females that produce only female offspring are an ephemeral phenomena in the evolution of stochastic gynodioecy and may not be observed in all populations (Frank 1989); however, if fitness differences among sexes is what maintains females in this species, either many populations will have cytoplasmic sex determination in which females produce all female offspring or all populations will be evolving toward a within-population equilibrium and female frequencies should be less each successive generation.)

2. By looking at the distribution of fitness traits among hermaphrodites, establish the possible existence and magnitude of a cost of restoration. If equilibrium models apply, the cost of restoration has the strongest affect on sex ratio in both simple and complex nuclear-cytoplasmic gynodioecy. Theory suggests that the cost of restoration is usually expressed through differences in pollen fitness (Delph et al. 2007); therefore, the possibility of cost and its maximum size can be estimated through a survey of hermaphrodites for pollen production and viability. Several protocols exist for these tests, which can be as simple as counting a known fraction of the total number of pollen grains under 100 × magnification and staining with simple dyes (Kearns and Inouye 1993).

Conclusions

Both genetic architecture and ecological context affect sex-ratio evolution. As we have seen for gynodioecy, whether nuclear and/or cytoplasmic genes determine sex has a large effect on equilibrium sex ratios. Further, the relative stability of populations determines whether equilibrium or stochastic patterns should be expected. Local ecology can affect the evolution of sex ratio both through effects on gene flow and colonization but also through sexual dimorphism and pollination efficiency. For biologists interested in the relative strength of genetic constraints and adaptation, gynodioecious systems offer an ideal model system in which a polymorphic trait with variable genetic architecture can be examined in replicate populations across landscapes.

Unlinked References

Barrett and Husband 1990, Ehlers et al. 2005.

Acknowledgements – We thank I. Anderson, A. Brothers, C. Herlihy and S. Scarpino for comments on the manuscript and acknowledge financial support from the NSF to L.F.D. (DEB 0210971).

Glossary

Compensation	a seed fecundity advantage of females as compared to hermaphrodites. In this paper, we will define x as the excess fecundity of females, such that if hermaphrodites have fecundity of 1, females have a fecundity of 1+x.
cost of restoration	a difference in fitness between classes of hermaphrodites caused by negative pleiotropic fitness effects of nuclear restorers of male fertility. In this paper, we will define *y* as the difference in fitness between hermaphrodites such that hermaphrodites without excess restorers (i.e. restorers not expressed in that individual's CMS background) have a pollen fitness of 1 and hermaphrodites with excess restorers have a pollen fitness of 1 −y.
cytoplasmic male sterility (CMS)	a syndrome of plant mitochondrial genes that causes pollen development to fail. Populations may contain several CMS types that can differ in their mechanism of pollen suppression.
nuclear gynodioecy	a type of gynodioecy in which the segregating variation for sex determining genes is nuclear.
nuclear-cytoplasmic gynodioecy	a type of gynodioecy in which the segregating variation for sex determining genes involves interactions between cytoplasmic male-sterility genes and nuclear restorers of male fertility.
restorers (nuclear restorers of male fertility)	nuclear alleles that suppress the phenotypic effect of corresponding CMS genes allowing the production of mature pollen. Because nuclear restorers are specific in their action such that they are only expressed in certain CMS backgrounds, there are multiple restorer loci and alleles in populations.
stochastic gynodioecy	a new term defined in this paper describing the type of gynodiocey when females are present in only a few isolated populations of a species. The lack of females in other parts of the species' range suggests that spatially and temporally stochastic metapopulation effects rather than population-level equilibrium effects explain the maintenance of females in these populations.
subdioecy	a breeding system intermediate between gynodioecy and dioecy but closer to the dioecy end of the continuum. In populations with this breeding system, female frequencies approach 50%. Because of the high frequency of strict seed producers, hermaphrodites gain nearly all their fitness via pollen but may still retain the possibility of reproducing through seeds.

UNCORRECTED PROOF

References

Ahmadi and Bringhurst 1991. **Please provide further details.**

Ashman, T.-L. 1999. Determinants of sex allocation in a gynodioecious wild strawberry: implications for the evolution of dioecy and sexual dimorphism. – J. Evol. Biol. 12: 648–661.

Ashman, T.-L. 2002. The role of herbivores in the evolution of separate sexes from hermaphroditism. – Ecology 83: 1175–1184.

Bailey 2002. **Please provide further details.**

Bailey, M. F. and McCauley, D. E. 2005. Offspring sex ratio under inbreeding and outbreeding in a gynodioecious plant. – Evolution 59: 287–295.

Bailey, M. F. et al. 2003. Modeling gynodioecy: novel scenarios for maintaining polymorphism. – Am. Nat. 161: 762–776.

Barr, C. M. 2004. Hybridization and regional sex ratios in *Nemophila menziesii*. – J. Evol. Biol. 17: 786–794.

Barrett, S. C. H. and Husband, B. C. 1990. The genetics of plant migration and colonization. – In: Brown, A. H. D. et al. (eds), Plant population genetics, breeding and genetic resources. Sinauer, pp. 254–277.

Caruso, C. M. et al. 2003. Gender-specific floral and physiological traits: implications for the maintenance of females in gynodioecious *Lobelia siphilitica*. – Oecologia 135: 524–531.

Charlesworth, B. and Charlesworth, D. 1978. A model for the evolution of dioecy and gynodioecy. – Am. Nat. 112: 975–997.

Charlesworth, D. 1981. A further study of the problem of the maintenance of females in gynodioecious species. – Heredity 46: 27–39.

Charlesworth, D. 1999. Theories of the evolution of dioecy. – In: Geber, M. A. et al. (eds), Gender and sexual dimorphism in flowering plants. Springer, pp. 33–60.

Charlesworth and Guttman 1999. **Please provide further details.**

Collin, C. L. et al. 2002. Natural enemies and sex: how seed predators and pathogens contribute to sex-differential reproductive success in a gynodioecious plant. – Oecologia 131: 94–102.

Couvet et al. 1986. **Please provide further details.**

Couvet et al. 1990. **Please provide further details.**

Couvet, D. et al. 1998. The maintenance of nucleocytoplasmic polymorphism in a metapopulation: the case of gynodioecy. – Am. Nat. 152: 59–70.

Darwin, C. 1877. The different forms of flowers on plants of the same species. – D. Appleton.

Delannay et al. 1981. **Please provide further details.**

Delph, L. F. 1990a. Sex-ratio variation in the gynodioecious shrub *Hebe strictissima* (Scrophulariaceae). – Evolution 44: 134–142.

Delph, L. F. 1990b. Sex-differential resource allocation patterns in the subdioecious shrub *Hebe subalpina*. – Ecology 71: 1342–1351.

Delph, L. F. 1993. Factors affecting intraplant variation in flowering and fruiting in the gynodioecious species *Hebe subalpina*. – J. Ecol. 81: 287–296.

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

Delph, L. F. 2003. Sexual dimorphism in gender plasticity and its consequences for breeding system evolution. – Evol. Dev. 5: 34–39.

Delph, L. F. and Wolf, D. E. 2005. Evolutionary consequences of gender plasticity in genetically dimorphic breeding systems. – New Phytol. 166: 119–128.

Delph, L. F. et al. 2007. Merging theory and mechanism in studies of gynodioecy. – Trends Ecol. Evol. 22: 17–24.

Dommée, B. et al. 1978. Natural selection and gynodioecy in *Thymus vulgaris* L. – Bot. J. Linn. Soc. 77: 17–28.

Eckhart, V. M. 1992. Spatio-temporal variation in abundance and variation in foraging behavior of the pollinators of gynodioecious *Phacelia linearis* (Hydrophyllaceae). – Oikos 64: 573–586.

Ehlers, B. K. et al. 2005. Sex inheritance in gynodioecious species: a polygenic view. – Proc. R. Soc. B 272: 1795–1802.

Fishman, L. and Willis, J. H. 2006. A cytonuclear incompatibility causes anther sterility in *Mimulus* hybrids. – Evolution 60: 1372–1381.

Frank, S. A. 1989. The evolutionary dynamics of cytoplasmic male sterility. – Am. Nat. 133: 345–376.

Godley, E. J. 1955. Breeding systems in New Zealand plants I. *Fuchsia*. – Ann. Bot. 19: 549–559.

Gouyon, P.-H. et al. 1991. Nuclear-cytoplasmic male sterility: single-point equilibria versus limit cycles. – Am. Nat. 137: 498–514.

Kaul, M. L. H. 1988. Male sterility in higher plants. – Springer.

Kearns, C. A. and Inouye, D. W. 1993. Techniques for pollination biologists. – Univ. Press of Colorado.

Kohn, J. R. 1989. Sex ratio, seed production, biomass allocation, and the cost of male function in *Cucurbita foetidissima* HBK (Cucurbitaceae). – Evolution 43: 1424–1434.

LaPorte, V. et al. 2001. The spatial structure of sexual and cytonuclear polymorphism in the gynodioecious *Beta vulgaris* ssp. *maritima*: I/ at a local scale. – Genetics 157: 1699–1710.

Lewis, D. 1941. Male sterility in natural populations of hermaphrodite plants. – New Phytol. 40: 56–63.

Lloyd, D. G. 1974. Theoretical sex ratios of dioecious and gynodioecious angiosperms. – Heredity 32: 11–34.

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

Lloyd, D.G. 1976. The transmission of genes via pollen and ovules in gynodioecious angiosperms. – Theor. Popul. Biol. 9: 299–316.

Manicacci et al. 1998. **Please provide further details.**

Marshall, M. and Ganders, F. R. 2001. Sex-biased seed predation and the maintenance of females in a gynodioecious plant. – Am. J. Bot. 88: 1437–1443.

Maurice, S. et al. 1993. The evolution of gender in hermaphrodites of gynodioecious populations with nucleo-cytoplasmic male-sterility. – Proc. R. Soc. B 251: 253–261.

UNCORRECTED PROOF

McCauley, D. E. and Taylor, D. R. 1997. Local population structure and sex ratio: evolution of gynodioecious plants. – Am. Nat. 150: 406–419.

McCauley, D. E. and Brock, M. T. 1998. Frequency-dependent fitness in *Silene vulgaris*, a gynodioecious plant. – Evolution 52: 30–36.

McCauley, D. E. et al. 2000. Population structure influences sex ratio evolution in a gynodioecious plant. – Am. Nat. 155: 814–819.

Murayama, K. et al. 2004. Variation of female frequency and cytoplasmic male-sterility gene frequency among natural gynodioecious populations of wild radish (*Raphanus sativus* L.). – Mol. Ecol. 13: 2459–2464.

Mutikainen, P. and Delph, L. F. 1996. Effects of herbivory on male reproductive success in plants. – Oikos 75: 353–358.

Oliveri et al. 1990. **Please provide further details.**

Olson, M. S. and McCauley, D. E. 2002. Mitochondrial DNA diversity, population structure, and gender association in the gynodioecious plant, *Silene vulgaris*. – Evolution 56: 253–262.

Pannell, J. 1997. The maintenance of gynodioecy and androdioecy in a metapopulation. – Evolution 51: 10–20.

Pannell, J. R. and Dorken, M. E. 2006. Colonisation as a common denominator in plant metapopulations and range expansions: effects on genetic diversity and sexual systems. – Landscape Ecol. 21: 837–848.

Richards, A. J. 1986. Plant breeding systems. – George Allen & Unwin, London.

Sakai, A. K. and Weller, S. G. 1999. Gender and sexual dimorphism in flowering plants: a review of terminology, biogeographic patterns, ecological correlates, and phylogenetic approaches. – In: Geber, M. A. et al. (eds), Gender and sexual dimorphism in flowering plants. Springer, pp. 1–31.

Schultz, S. T. 1994. Nucleo-cytoplasmic male sterility and alternative routes to dioecy. – Evolution 48: 1933–1945.

Stevens, D. P. and Van Damme, J. M. M. 1988. The evolution and maintenance of gynodioecy in sexually and vegetatively reproducing plants. – Heredity 61: 329–337.

Taylor, D. R. et al. 1999. Ecological genetics of gynodioecy in *Silene vulgaris*: relative fitness of females and hermaphrodites during the colonization process. – Evolution 53: 745–751.

Taylor, D. R. et al. 2001. A quantitative genetic analysis of nuclear-cytoplasmic male sterility in structured populations of *Silene vulgaris*. – Genetics 158: 833–841.

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

UNCORRECTED PROOF