PARENTAL EFFECTS ON PROGENY PHENOTYPE IN PLANTS: DISTINGUISHING GENETIC AND ENVIRONMENTAL CAUSES

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Abstract.—The experimental measurement of additive genetic variation in plant populations is complicated by the potential for non-Mendelian inheritance. Maternal influences on progeny phenotype resulting from the cytoplasmic inheritance of plastids or RNA transcripts and effects of the maternal environment have consequently been the focus of much research. To exclude or to control for these sources of variation, breeding designs (e.g., cross-factored, nested, or diallel) in which genetically unrelated pollen donors are mated to maternal genotypes have been adopted. Using these designs, some empirical studies have detected statistically significant differences among pollen donors in the mean performance of their pollen (the mature male gametophytes) or in the mean phenotype of their progeny. These statistical effects of pollen-donor identity on pollen performance or progeny phenotype have frequently been interpreted as evidence for additive genetic variance among pollen donors, although patrilineal cytoplasmic inheritance or effects of the paternal environment on pollen performance or gene expression are recognized as alternative explanations. We note that environment-specific selection among developing gametophytes-in which the environment experienced by developing pollen grains (or ovules) provides a selective force causing the differential survival of gametophyte genotypes (analagous to meiotic drive)—is an additional process that may cause genetically based paternal (or maternal) effects on gametophyte performance. If genes selected during this process are expressed in the sporophyte (postfertilization), this process could also influence the phenotype of the diploid progeny. Here, we review the potential causes of statistically significant differences in mean phenotype among the gametophytes or progeny of maternal (seedbearing) or paternal (pollen-donating) parental plants. We suggest an experimental approach that permits the detection or elimination of selection among developing gametophytes as one such cause. Specifically, the replication of homozygous parental genotypes within and across environments allows the detection and measurement of paternal and maternal environmentally induced effects on gametophyte or offspring phenotype, while eliminating meiotic drive as a source of the phenotypic variation.

Key words.—Additive genetic effects, diallel, gametophytic selection, maternal effects, meiotic drive, non-Mendelian inheritance, parental effects, pollen performance.

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Evolutionary ecologists typically assess the degree of genetic variation in fitness-related traits in order to evaluate the potential for natural selection, genetic drift, and gene flow to drive genetic change. Until approximately 1986, the most common method used to detect genetically based variation in plants was to cultivate progeny representing distinct maternal genotypes (or maternal lineages), clones, or ecotypes in a uniform environment. Estimating the magnitude of genetically based variation in fitness-related traits using this method, however, has been widely recognized as problematic. Quantitative and ecological geneticists have identified several potential causes (in addition to additive, Mendelian, genetic variation) of differences among maternal (seed-bearing) genotypes with respect to the phenotype of their progeny, and each of these has distinct evolutionary implications (Antonovics and Schmitt 1986; Roach and Wulff 1987; Jolls and Chenier 1989; Lyons et al. 1989). For example, matrilineal inheritance of fitness-related traits resulting from the expression of cytoplasmic genes can provide time lags for or constraints to the evolution of phenotypic traits and even cause them to evolve in the opposite direction of selection. Because maternally transmitted nonnuclear genes can influence the rate and direction of the response of nuclear genes to selection, the measurement of non-Mendelian maternal effects on progeny phenotype and fitness are necessary for

accurate predictions of evolutionary trajectories (Kirkpatrick and Lande 1989; Lande and Kirkpatrick 1990).

Matrilineal inheritance in plants can take several forms. Even small differences among the environments in which maternal plants are raised can create strong phenotypic differences among their progeny, particularly in traits expressed early in the life cycle (Schaal 1984; Alexander and Wulff 1985; Parrish and Bazzaz 1985; Wulff 1986a,b,c; Stratton 1989; Aarssen and Burton 1990). In addition, genes in the endosperm, seed coat, and integument, maternally derived extranuclear genes, and the abiotic maternal environment can mask the expression of nuclear genes in the progeny. Consequently, predictions of the rate of evolutionary change in traits expressed solely by the nuclear genome require estimates of additive genetic variance (V_a) or narrow-sense heritability (h^2) that exclude or control for these sources of variation.

Although the measurement of the narrow-sense h^2 of fitness-related traits is much more labor intensive than analagous measures of broad-sense H^2 , many evolutionists have provided quantitative or qualitative estimates of narrow-sense h^2 for such traits in natural plant populations (Zamir et al. 1981; Antonovics and Schmitt 1986; Marshall and Ellstrand 1986; Mazer et al. 1986; Schmitt and Antonovics 1986; Mazer 1987a,b, 1989; Weis et al. 1987; Marshall 1988, 1991; Marshall and Whittaker 1989; Nakamura and Stanton 1989; Pittmann and Levin 1989; Andersson 1990; Cruzan 1990;

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TABLE 1. Mechanisms that could produce maternal or paternal effects in the statistical sense with demonstrated or hypothetical examples. Mechanisms that are italicized represent non-Mendelian maternal or paternal effects as commonly used in quantitative genetics. See the text for explanations and references.

Mechanism	Maternal example	Paternal example
1. Additive genetic variance 2. Nonadditive genetic variance	Chromosomal genes	Chromosomal genes
a. Dominance, epistasis	Chromosomal genes	Chromosomal genes
b. Cytoplasmic inheritance	Mitochondrial genes, chloroplast genes, and RNA transcripts in surviving megaspore	Mitochondrial genes, chloroplast genes, and RNA transcripts in pollen grain
3. Effects of parental environment	Seed size influenced by resource avail- ability	Pollen performance influenced by re- source availability
4. Environmentally induced gene expression	Temperature-mediated gene expression in the seed coat	Environmentally induced gene expres- sion in pollen
5. Environment-specific gametophytic selec- tion (environment-dependent meiotic drive)	Selection among eggs or megaspores	Prepollination selection among micro- spores or mature pollen grains

Schwaegerle and Levin 1990; Snow 1990; Venable and Burquez 1990; Young and Stanton 1990; Biere 1991a,b; Fenster 1991; Mazer and Schick 1991; Morse and Schmitt 1991; Richardson and Stephenson 1991). The primary aims of these studies, which represent a wide array of breeding designs, were to examine the performance of progeny of genetically distinct pollen donors in order to determine the following: (1) the degree to which natural populations express V_a in fitness-related traits and (2) whether strong nonadditive maternal effects on progeny fitness reduce narrow-sense h^2 by contributing to total phenotypic variance, thereby constraining evolutionary change of the nuclear genome. Although these studies have identified or reduced certain biases in heritability estimates, other biases, such as those resulting from inbreeding depression and the small number of genotypes typically sampled, are also now widely recognized (Gebhardt 1991). The awareness of these biases has led to many improvements in the statistical methods used for estimating the narrow-sense heritability of quantitative traits (Thompson and Shaw 1990, 1992; Shaw 1991, 1992; Shaw and Mitchell-Olds 1993).

Whereas estimates of V_a and narrow-sense h^2 based on the transmission of phenotype from paternal plants to their offspring are likely to be less biased than those based on maternal transmission (Hayman 1954; Griffing 1956; Cockerham and Weir 1977), there is growing evidence that progeny phenotype may be subject to paternal environmental and extranuclear effects in the same manner that it is subject to such maternal effects. We are motivated by several recent empirical studies that have detected statistically significant differences among the gametes or progeny of distinct pollen donors (paternal effects in the statistical sense, defined below). We suggest that these observations may be as difficult to interpret in terms of their evolutionary role as are phenotypic differences among maternal sibships. The results of these studies have not previously been brought together to raise collective doubt about the robustness of narrow-sense heritability estimates.

We have four objectives. First, we wish to identify and to distinguish among the potential causes of differences among the gametophytes or diploid progeny produced by different pollen donors and maternal plants. We discuss one possible cause—environment-specific meiotic drive (that is, environment-specific selection among developing gametes or gametophytes) that to our knowledge has not been recognized as a source of variation among pollen donors. Second, we wish to point out that some of these alternative causes produce identical results with respect to phenotypic differences among pollen donors or paternal sibships. Third, we review the results of a few recent studies of the influence of pollendonor identity on gamete or progeny phenotype that may be more ambiguous than generally recognized. Finally, we suggest that the use of homozygous lines as pollen-donor genotypes can eliminate environment-dependent selection among developing gametophytes (meiotic drive) as a source of variation among donors in the performance of their pollen or in the mean phenotype of their diploid progeny. In addition, the use of replicated inbred lines for each donor genotype can allow the manipulation and measurement of macro- and microenvironmental variation as sources of paternal effects on progeny phenotype.

Potential Causes and Consequences of Maternal Effects (and Analogous Paternal Effects) on Gametophyte or Progeny Phenotype

Phenotypic differences among the haploid gametophytes or diploid progeny of different maternal or paternal (pollendonor) plants can be caused by five phenomena, each with distinct consequences for the process of evolution by natural selection (Table 1). To avoid confusion, it is important to distinguish two ways of defining or identifying parental (maternal or paternal) effects on gametophyte and progeny phenotype. First, analyses of variance in which maternal or paternal identity are included as main effects may detect statistically significant differences among maternal or paternal gametophyte or offspring means in a quantitative trait. Such effects of parental identity on offspring phenotype are parental effects in a statistical sense. This kind of parental statistical effect on progeny phenotype specifies no particular cause for the observed differences among parental or sibship means; the differences may or may not be heritable. Moreover, differences among maternal versus paternal sibships may or may not be symmetrical; for example, in a bisexual species, an analysis of variance (ANOVA) of a diallel cross

may detect statistically significant maternal effects on progeny phenotype but no paternal effects.

Second, as commonly used by quantitative geneticists, parental effects may refer specifically to traits that are asymmetrically inherited (i.e., the transmission of phenotype depends on the gender of the parent). For example, the term "maternal effects" in plants refers to effects on offspring phenotype transmitted by a plant only to the seeds that it bears (and not to the seeds it sires on other plants). Such maternal effects may have a genetic basis (resulting from genes passed on through the cytoplasm of the egg cell; Lande and Kirkpatrick 1990, and references therein); they may be due solely to the environmental conditions in which the plant is developing (i.e., maternal environmental effects; Falconer 1989); or they may represent the outcome of a maternal genotype-by-environment interaction (reflecting both genetic and environmental factors). Such maternal effects are always considered to be asymmetrical (i.e., not transmitted through the pollen, even in bisexual species). Extending this definition to paternal effects, we can imagine that an allele may influence the mean phenotype of the progeny sired by the genotype that bears it but that the effect on progeny phenotype is not transmitted to the seeds produced by this genotype as a maternal plant. We distinguish between these two definitions of parental effects as necessary, as we outline the five phenomena.

1. Additive Genetic Variation among Parental Plants

Additive genetic variation among maternal individuals in a trait may account for mean phenotypic differences among groups of the female gametophytes or diploid seeds they produce. This type of maternal "genetic" effect will be detected in an ANOVA as described above (the statistical definition of a maternal effect), and it represents genetic variation that will also be transmitted to progeny sired by these maternal plants if they are bisexual. When seed-bearing genotypes differ in expected fitness, we predict evolutionary change between generations in the nuclear-gene frequencies of independently evolving fitness-related traits that exhibit significant V_a .

Differences in the nuclear genes transmitted by the pollen of distinct paternal genotypes may similarly cause phenotypic differences among their gametophytes or diploid progeny with respect to pollen germination rates (i.e., the delay between pollen deposition and germination, or the probability of germination), pollen-tube growth rate, fertilization rate, the probability of seed abortion, final seed mass, the probability of seed germination, and any measurable character of the progeny (Quesada et al. 1991). Breeding designs commonly used to detect such paternal effects on the phenotypes of diploid progeny include the North Carolina design II and the diallel cross (Henderson 1952; Hayman 1954; Griffing 1956; Cockerham and Weir 1977; Mather and Jinks 1982).

2. Nonadditive Genetic Variation among Parents

Nonadditive genetic variation resulting from dominance or epistatic interactions among alleles expressed in a maternal plant's polyploid endosperm or diploid progeny can create phenotypic differences among maternal sibships. If this phenotypic effect were to be transmitted through a bisexual plant's pollen as well, then it would not be a maternal effect as specified by the second definition provided above (i.e., asymmetrical). Similarly, nonadditive effects of genes expressed by maternally inherited cytoplasmic organelles may contribute to variance among maternal sibships in progeny phenotype and even influence the evolutionary trajectory of nuclear genes (Kirkpatrick and Lande 1989; Lande and Kirkpatrick 1990). More complex sources of nonadditive genetic variation include interactions between nuclear and cytoplasmic genes that contribute to quantitative variation in progeny phenotype or performance (for examples that seek empirical evidence of these interactions in plants, see Antonovics and Schmitt 1986, and Schwaegerle and Levin 1990).

In bisexual species, nonadditive sources of variation may create phenotypic differences between an individual's offspring produced through seed production (the maternal sibship it produces) and those produced through pollen (the paternal sibship it produces). For example, cytoplasmic genes could be gender specific in their effect. If cytoplasmic genes exist that influence the nutrient-garnering ability of endosperm tissue (where they may be present in multiple copies), then such genes may affect the seed mass of the maternal sibships in which they are expressed but not the seed mass of the paternal sibships to which they are transmitted.

Variation among pollen donors resulting from dominance or epistastis among nuclear or cytoplasmic genes may also have measurable effects on progeny phenotype (Milligan 1992). These genetic interactions may be expressed within the pollen cytoplasm alone (in the case of epistasis), or they may occur after fertilization, involving both parental genomes. Interactions between paternal nuclear genes and the zygote cytoplasm may also contribute to phenotypic differences among paternal half sibships. Strong statistical interactions between maternal and paternal genomes and strong reciprocal effects on progeny phenotype detected by the AN-OVAs of diallel or cross-factor breeding designs can be evaluated in some cases to isolate and to estimate the magnitude of some of these sources of genetically based variance (Antonovics and Schmitt 1986). Finally, although Lande and Kirkpatrick (1990) do not emphasize this in their theoretical work, their results imply that the expression of extranuclear paternal genes could influence the evolution of nuclear genes expressed after fertilization.

Extranuclear (or extraembryo) paternal genetic effects on progeny phenotype are generally considered to be of less importance (rarer and less easily detectable) than extranuclear maternal genetic effects because of the lower volume of pollen cytoplasm relative to egg cells, the absence of plastids in the generative or sperm cells of many taxa (Corriveau and Coleman 1988, p. 1443), and the relatively low dose of paternally derived genes present in endosperm cells. However, there is much evidence that nonnuclear paternal genes are not infrequently transmitted to the egg sac. For example, Corriveau and Coleman (1988) detected "putative plastid DNA" in the generative or sperm cells of pollen from 43 species (26 genera representing 15 families) of 235 investigated species; Szmidt et al. (1987) detected paternal inheritance of chloroplast DNA in Larix; and paternal inheritance of plastids in Medicago sativa has been widely observed (Schumann and Hancock 1989). Sewell et al. (1993) found

that 2.9% and 11.1% of the progeny of congeneric hybrids within *Liriodendron* and *Magnolia* (Magnoliaceae), respectively, contained uniparental, paternally transmitted plastids.

The growing literature documenting paternal and biparental inheritance of plastids in wild and domesticated plants suggests that this kind of paternal influence on progeny phenotype may have been unfairly discounted in many quantitative-genetic studies (Lombardo and Gerola 1968; Kirk and Tilney-Bassett 1978; Hagemann 1979; Cass 1983; Medgyesy et al. 1986; Chiu et al. 1988; Cornu and Dulieu 1988; Corriveau and Coleman 1988, 1990; Corriveau et al. 1989; Hagemann and Schroder 1989; Schumann and Hancock 1989; Smith 1989; Boblenze et al. 1990; Horlow et al. 1990; Masoud et al. 1990; White 1990; Zhu et al. 1990, 1991; Hause 1991; Shi et al. 1991; Derepas and Dulieu 1992; Tilney-Bassett et al. 1992; Kuroiwa et al. 1993; Amoatey and Tilney-Bassett 1994; Sodmergen et al. 1994). Note, however, that the transmission of nonnuclear genes through sperm does not guarantee a paternal effect on progeny phenotype unless these genes have strong phenotypic effects.

3. Resource Availability: Parental Effects Resulting from the External Environment

The availability of resources to a maternal plant may influence progeny phenotype due to the quantity or quality of nutrients available for provisioning offspring. Seed-bearing plants growing in resource-rich environments frequently produce larger or higher quality seeds than those growing in resource-poor environments (Stratton 1989; Miao et al. 1991a; Mazer and Wolfe 1992) or have different patterns or rates of seed abortion (Marshall and Ellstrand 1988). Seed size may also be influenced by the temperature of the maternal environment, apparently through its effect on respiration rates (Potvin and Charest 1991). Maternal environmental effects on progeny performance have been found in some cases to be transmitted for several generations (Durrant 1962a,b, 1971; Hill 1965; Wulff and Alexander 1985; Miao et al. 1991b), although the effect is sometimes reversible (Durrant and Jones 1971).

If the strength of this kind of environmental effect depends on the nuclear genotype of a maternal plant (a genotype-byenvironment interaction), natural selection among maternal genotypes in a heterogeneous environment may result in gene frequency changes in genes influencing the plastic response to environmental enrichment as well as in genes influencing the mean phenotype. For example, if some maternal genotypes produce higher quality or more offspring than others when exposed to enriched environments, then the frequency of the former genotypes may increase following nutrient pulses.

Resource-related maternal environmental effects are one source of environmental variance (V_e) . When V_e is high, and elevates total phenotypic variance (V_p) in a given trait, it will reduce narrow-sense heritability (V_a/V_p) and limit the response to selection on the trait. Because maternal environmental effects provide no phenotypic variance on which selection may act directly to cause evolutionary change, maternal environmental effects are generally considered to constrain or to retard the evolution of nuclear genes. It is interesting to note, however, that there is evidence that environmental conditions can cause significant changes in the quantity and quality of nuclear DNA within a single generation. The long-term evolutionary consequences of this environmental effect have not been explored (Evans et al. 1966; Evans 1968; Durrant and Jones 1971).

From the paternal perspective, pollen produced in one environment may receive a higher level of mineral nutrition, water, or other limiting resource than pollen produced elsewhere. If the quality of the paternal environment influences pollen size or nutrient allocation, and if these attributes influence pollen performance (e.g., pollen germination, pollentube growth, the ability to reach, find and fertilize ovules, or the ability to avoid abortion), then pollen produced in different environments may achieve different levels of reproductive success even if each environment contributes a genetically identical pollen pool. Such paternal environmental effects differ from maternal environmental effects because only the male gametophyte is affected; in contrast, maternal environmental effects may act on any of the maternally derived tissues within a developing seed: the megagametophyte, seed coat, endosperm, embryo, or megasporophyte tissue.

Putative paternal environmental effects on pollen quality or competitive ability have been identified in *Pennisetum clandestinum*, *Cucurbita pepo*, and *Raphanus sativus* (Younger 1961; Schlicting 1986; Young and Stanton 1990; Lau and Stephenson 1993); however, it is not clear whether the observed phenotypic effects of environmental stress were mediated by a genetic change (e.g., altered gene expression or selection among developing gametophtyes; see below). Phenotypic effects associated with environmentally induced changes in nuclear DNA have been reviewed by Cullis (1986), supporting this possibility.

4. Environmentally Induced Differences in Parental Gene Expression

Given that environmental factors can influence enzyme activity, then gene expression within germ lines and their derivative tissues may be sensitive to the environmental conditions in which they develop. For example, the environment experienced by a maternal plant may affect gene expression within its seed coats, egg cells, embryos, and developing endosperm. This environment-specific gene expression may in turn influence the phenotype of subsequently fertilized ovules and developing embryos. The result would be an environmentally induced maternal effect on progeny phenotype that is statistically identical to the effect of environmental enrichment or depletion, but in this case the maternal effects on progeny phenotype would have been due to differences among ovules in gene expression. The ability of the maternal environment to influence gene expression among progeny in subsequent generations is suggested by the work of Alexander and Wulff (1985), whereas the general phenomenon of environment-dependent gene expression is supported by the observation that herbivore resistance may be induced in seedlings exposed to herbivory (Karban and Myers 1989).

Similarly, if the paternal environment affects gene expression in the male gametophyte, then pollen produced in one environment may be phenotypically distinct from pollen produced in another environment, even if both pollen sources initially contained identical nuclear genomes. In the absence of enzymatic or molecular assays to detect differences among gametophytes or progeny in gene expression, it may not be possible to distinguish between (3) and (4) as environmental causes of parental influences on gamete or offspring phenotype.

5. Environment-Specific Selection Regimes among Gametophytes during Development: Prepollination Selection among Gametic Genotypes

Finally, consider that different external environments may impose distinct selective regimes on populations of gametes or gametophytes. For example, different maternal environments may each impose a unique selective regime on the eggs and ovules developing within them. This environmentspecific natural selection could result in a process in which the gene frequencies among viable unfertilized egg cells depend upon environmental conditions (environment-dependent meiotic drive). This process has not been clearly identified in wild plant species (but see Casper 1988; Marshall 1988).

Similarly, if the environment in which male gametophytes develop imposes a distinct selective regime on the developing pollen grains, then pollen produced in one environment may be genetically distinct from that produced in another environment. If selection among developing male gametophytes is sufficiently strong and environment-specific, then the genetic composition of the mature pollen pools will depend upon the paternal environment, potentially resulting in phenotypically and genetically distinct paternal sibships. A cross-generational effect of environment-specific gametophtye selection requires that those genes under selection are either expressed, or genetically linked to genes that are expressed, in the resulting sporophyte generation. This type of gametophytic selection" should not be confused with postpollination selection among pollen genotypes, which might also occur with environment-specific outcomes.

Unlike environmentally induced gene expression (case 4), in which all genotypes may survive, environment-specific gametic or gametophytic selection will purge the pollen pool of particular genotypes, resulting in pollen gene frequencies that differ among environments. If the nature of this kind of selection is gender-specific (affecting gene frequencies among eggs differently than those among pollen grains), then the relationship between maternal phenotype and offspring phenotype (i.e., the mother-offspring regression) may differ from that between paternal and offspring phenotypes. In other words, parental effects on offspring genotype may differ between the genders of a bisexual individual or between the sexes in dioecious taxa.

This last potential cause of parental effects on progeny phenotype requires three elements to explain phenotypic differences among diploid sibships produced by parents raised in distinct environments: selection among developing gametes or gametophytes, Mendelian inheritance, and an association between the favored (or disfavored) gametophyte genotype and sporophyte phenotype. It does not, however, require extranuclear inheritance or environmentally induced effects (sensu cases 3 or 4, above) on offspring phenotype. Whether it is a more frequent or parsimonious explanation for statistical parental effects on progeny phenotype, however, remains a question for empirical research.

Consequences for Studies of Paternal Influences on Progeny Phenotype

Although each of the potential sources of maternal effects on progeny phenotype discussed above has an analog in paternal transmission of phenotype and genotype, this has not been widely recognized in the quantitative-genetics literature. Quantitative evolutionary geneticists generally interpret statistically significant paternal effects on progeny phenotype (when pollen donors are mated with a random or shared array of maternal plants) as evidence for the presence of additive genetic variation among pollen donors in the observed trait(s). If case (5) is a common phenomenon, however, then differences among paternal sibships may simply be the result of natural selection having occurred within one or several pools of male gametes or gametophytes.

The process of male gametophytic selection during development may be a cause of apparent paternal environmental effects on offspring phenotype, particularly for traits expressed during relatively late stages in the life cycle of the offspring generation. That is, traits expressed early in the sporophyte stage of the life cycle (e.g., seed size, seed viability, seed dormancy, germination rate) may more likely be subject to strong maternal environmental influences, potentially swamping out paternal effects that might act on early offspring traits. If selection among male gametophytes influences offspring genotype, perhaps only those traits expressed relatively late in the life cycle will show its effects (i.e., traits that are relatively free from maternal environmental influences).

Studies Detecting Paternal Effects on Offspring Phenotype

Many phenomena can create groups of pollen or of progeny (paternal sibships) that are phenotypically distinct from each other: additive genetic variation among pollen donors; the paternal transmission of nonadditive genes; extranuclear organelles or RNA transmitted through pollen; environmental differences in resource availability; environmentally induced gene expression; and environment-specific selection among male gametophytes. Consequently, the use of cross-factor breeding designs that aim to detect additive genetic variation among pollen donors by mating them with a random array of maternal plants provide ambiguous results. This is also the case for nested designs in which each of many pollen donors is mated with a distinct group of unrelated maternal plants. To illustrate this ambiguity, we review several recent studies that provide quantitative or qualitative measures of additive genetic variation among pollen-donor genotypes but which might reflect environmentally induced gene expression or environment-specific selection. We then offer some suggestions for breeding designs that may reduce the degree of ambiguity.

Several studies have detected statistically significant differences among the progeny of different pollen donors with respect to seed development, life history, or morphological traits. Investigators often infer that these paternal effects indicate the presence of additive genetic varation in these traits and the potential for natural selection to direct their evolution. For example, in a study of paternal effects on seed size in Crepis tectorum (Asteraceae), Andersson (1990) crossed parental plants in a nested breeding design (each of 100 pollen donors successfully pollinated two unrelated maternal plants). A one-way ANOVA detected significant differences among paternal half-sib groups with respect to seed width and seed weight. Pollen-donor identity influenced the mean values of these traits, partly resulting from the following indirect effect: pollen donors differed in the probability of seed set, and the inverse relationship between seed number per flower head and mean individual seed mass resulted in an effect of pollen-donor identity on seed mass. Andersson logically argued that the presence of genetic variance suggested by the significant paternal effects on progeny phenotype indicated that genetic variation in seed mass is being maintained in the study population of C. tectorum by factors other than natural selection directly on seed mass. Two potential problems arise in the interpretation of these data. First, because only two maternal plants were used per donor, it is possible that strong maternal effects on seed set and seed mass created apparent differences between paternal sibships that had little to do with donor genotype. Second, if the observed paternal effects on seed set are due to extranuclear genes, to environmentally induced gene expression among pollen donors, or to gametophytic selection acting differently within different donors before pollen maturation, then they reflect something other than additive genetic variation.

Similarly, the series of cross-factor and nested breeding designs performed on Raphanus raphanistrum and R. sativus (Mazer, et al. 1986; Mazer 1987a,b; and Mazer and Schick 1991) were designed in part to detect and to estimate the level of additive genetic variation in life-history and fitnessrelated traits. In the cross-factor designs, pollen donors were raised under relatively uniform greenhouse conditions, and each donor was mated with the same four maternal plants. In the nested design (Mazer and Schick 1991), each donor pollinated a different group of four unrelated maternal plants; the use of four plants per donor (compared to two per donor) reduced the probability that significant phenotypic differences between paternal sibships were due to non-Mendelian maternal effects (operating in the recipients). These studies followed the norm of interpreting statistically significant paternal effects on progeny phenotype as evidence for significant additive genetic variation in traits such as mean seed weight, germination rate, flowering date, fruit set, and subsequent reproductive components. In light of the above alternate causes of statistically significant paternal effects on progeny phenotype, however, we would suggest that this interpretation is equivocal. Just as it has been observed that greenhouse-raised maternal plants can exhibit nonheritable effects on offspring traits (e.g., seed mass; Mazer 1987b), it is possible that greenhouse-raised pollen donors do so as well.

In a novel effort to detect environmentally induced paternal effects on gametophyte performance analogous to maternal effects on seed development and progeny phenotype, Young and Stanton (1990) experimentally manipulated the paternal environment in *R. sativus*. Their aim was to determine whether environmental differences during pollen maturation [in this

case, a "stressed" treatment (no nutrient supplementation during growth) vs. a "control" treatment (supplemented 2–3 times weekly with Hoagland's solution) in the greenhouse] could influence pollen performance following single-donor and mixed-donor pollinations. They found statistically significant effects of the paternal environment on the number of seeds sired when pollen-donor genotypes competed within styles for access to ovules. They point out that most investigators have equated paternal effects on pollen performance or progeny phenotype with heritable genetic variation; theirs is the first study to demonstrate that even a subtle manipulation of the environment in which pollen is produced can affect the relative reproductive success of different pollen genotypes.

Young and Stanton (1990, p. 1663) propose that their study "demonstrates the existence of strong nongenetic components" to paternal reproductive success. One limitation of the study (noted by the authors), however, was that, because of the self-incompatibility of R. sativus, it was not possible to replicate pollen-donor genotypes among environments. Instead, pairs of full sibs were used to create genetically similar (but not identical) groups of plants subject to the environmental treatments. We suggest that there may in fact be a genetic component to the differences that they observed in the competitive ability of pollen produced by donors that developed in different environments. For example, if the presence, intensity, or direction of selection among male gametes or gametophytes during pollen development differed between their treatments, then it is possible that the genetic composition of the pollen produced in the two environments differed. If, under control conditions, alleles associated with relatively high postpollination pollen performance (e.g., increased pollen-tube growth rate) were selectively favored during pollen development, whereas under stressed conditions such alleles were selectively neutral or selected against, this environment-specific selection regime could account for the observation that pollen produced under stressful conditions performed relatively poorly. When highly heterozygous pollen-donor genotypes (which are the norm in *R. sativus*) are exposed to contrasting environments, differences in pollen performance among the donors raised in each environment may have a genetic basis.

Breeding Program to Rule Out Environment-Specific Selection during Pollen Development as a Cause of Paternal Effects on Progeny Phenotype: The Use of Homozygous Lines

Because paternal influences on seed development can conceivably be due to any of the genetic influences and interactions reviewed above, the breeding designs that have been used by plant evolutionary geneticists do not allow unbiased estimates of additive genetic variation among pollen donors. We suggest an alternative breeding design that may be used: (1) to determine whether environment-specific gametophytic selection during pollen development may create significant differences among pollen donors (within or among imposed environmental treatments) in the performance or phenotype of their pollen or progeny; (2) to detect environmentally induced paternal effects on offspring phenotype independent of environment-specific selection or meiotic drive; (3) to examine the symmetry of maternal and paternal effects within environments; and (4) to determine whether the environmental induction of maternal effects is more likely than the induction of paternal effects. We do not specify here the details of the ANOVAs necessary for significance testing.

Our primary point is that the use of homozygous genotypes (inbred lines) as pollen donors can eliminate environmentspecific gametophytic selection during pollen development as a possible cause of differences among donors in the phenotype of their pollen or diploid progeny. This is simply because, in the absence of mutation, homozygous individuals will produce pollen grains that are all genetically identical. If homozygous genotypes are replicated and raised in different environments or replicated within a uniform environment, then major differences among replicates in pollen or progeny phenotype cannot be due to environment-specific selection among developing gametophyte genotypes. Macroor microenvironmental differences in gene expression or resource availability would be the remaining explanations.

Comparing Homozygous and Heterozygous Lines across Environments

The comparison of the environmental response of homozygous relative to heterozygous lines (wild type or produced through the hybridization of homozygous lines) may allow the detection of environment-specific meiotic drive. For example, consider a comparison of the performance and phenotype of pollen (and progeny) produced by homozygous genotypes that are cloned (i.e., replicated) and raised in different environments with the pollen (and progeny) produced by heterozygous genotypes cloned and raised in the same set of environments. One might observe environmental effects among replicates of the heterozygous lines but not among homozygous replicates (a heterozygosity-by-environment interaction). One explanation for the differential response of heterozygotes would be that there are paternal environmental effects on pollen or progeny phenotype mediated by environment-specific meiotic drive. If homozygous and heterozygous lines respond identically to environmental variation, then environment-specific selection among developing gametes or gametophytes could not be causing the environmental effect. If homozygous lines exhibit an environmental response whereas heterozygous lines do not, one interpretation would be that the heterozygous condition provides a homeostatic effect on phenotype, which may or may not be generated by meiotic drive in one or both environments.

Microenvironmental versus Macroenvironmental Paternal Effects

Parental environmental effects on progeny phenotype may be classified as belonging to one of two types: microenvironmental or macroenvironmental. Even when individuals are raised in what is intended to be a uniform environment, nonheritable maternal effects on progeny phenotype may be generated (e.g., Mazer 1987b). These may be attributed to small microenvironmental differences between individuals that influence progeny phenotype, particularly for traits expressed during early portions of the offspring life cycle. The potential for microenvironmental variation alone to induce phenotypic differences among the progeny of different maternal plants prevents the precise measurement of extranuclear genetic effects on progeny phenotype. To our knowledge, microenvironmental paternal effects on pollen performance or progeny phenotype have not been directly measured in plants. Macroenvironmental variation can be defined as that caused by intentional or measurable environmental attributes (e.g., water stress, CO_2 concentration, or nutrient quality).

The replication of homozygous lines within and among controlled environmental treatments would allow the detection of micro- and macroenvironmental paternal effects on pollen performance or progeny phenotype that are not due to environment-specific selection among developing gametophyte genotypes. As described above, however, it will not be possible to determine whether observed differences among genetically identical homozygous pollen donors are due directly to resource availability or to environment-specific gene expression (cases 3 and 4).

Maternal versus Paternal Environmental Effects

Matings among homozygous lines may also be useful to assess the relative importance of maternal and paternal environmental influences on progeny phenotype. The pollination, by a donor raised in one environment, of homozygous, identical maternal plants raised in an array of environments will allow the detection of environmentally induced maternal effects (cases 3 and 4, above) ruling out the possibility of environment-specific meiotic drive among megagametophytes. If genetically identical homozygous pollen donors, raised in distinct environments, are compared with respect to their pollen performance or the mean phenotype of their offspring, one may detect environmentally induced paternal effects (cases 3 and 4, above). If a number of homozygous lines (each replicated within and among controlled environments) are evaluated as both maternal and paternal parents, the relative magnitude of maternal and paternal environmental effects on gametophyte performance or progeny phenotype can be determined.

If a complete diallel cross is conducted, using homozygous parents, in each of several environments, additional questions may be addressed. Each diallel may be subject to its own ANOVA, and the mean squares (and P-values) associated with maternal and paternal effects compared among diallels. Given similar sample sizes and balanced designs, such comparisons will determine whether the relative or absolute magnitude of maternal or paternal effects (or their interaction) is environment-specific. Alternatively, a three-way ANOVA that includes donor effects, recipient effects, diallel (environment) effects, and their interactions, could detect significant environment-specific donor effects (i.e., if there is a significant donor-by-diallel interaction). When homozygous lines are used to construct the diallels, then environmentspecific gametic selection cannot be a cause of observed differences between environments in the magnitude or nature of parental effects.

Limitation of the Use of Homozygous Lines as Pollen Donors

One major limitation of the use of homozygous lines as alternative pollen donors is that inbreeding depression, link-

age disequilibrium generated by inbreeding, or the use of homozygous mates in species that normally outcross, bias narrow-sense heritability estimates (e.g., Oakes 1967; Gebhardt 1991). Therefore, homozygous lines cannot be used to generate accurate h^2 estimates. Rather, their use (and comparison with heterozygous lines) can determine whether, for a given species, environment-specific meiotic drive may account for differences observed between normally heterozygous pollen donors. If so, then estimates of additive genetic variance derived from intersire variance components (cf. the diallel, North Carolina II, and nested designs) are equivocal (at least for that species).

The availability of homozygous lines will determine the practicability of these suggestions. In self-compatible species, these can be developed by repeated selfing. However, for self-incompatible species, it would be necessary to induce autodiploidy of gametophytic tissue or to force selfing for several generations (as can be done in some species via hand pollinations within flower buds). There may be problems in extrapolating results from such autodiploids and forced selfings to natural populations.

Replication of Pollen Donors and Recipients from Seed versus from Vegetative Clones

Adult plants representing a given homozygous line do not necessarily bear identical complements of cytoplasmic genes, even if such plants are identical with respect to their nuclear genes. In angiosperms, a process that may generate such differences within a homozygous line is the unequal division, during meosis of the megaspore mother cells, of cytoplasmic genes among the resulting megaspores. If this occurs, then embryo sacs within a maternal plant may differ in the cytoplasmic constitution of their surviving megaspore. In this case, embryos developing within a maternal plant could differ in their complement of cytoplasmic genes, and the F_1 adults into which they grow may produce germ lines that also differ cytoplasmically.

In contrast, a vegetatively cloned genotype should generate ramets, through mitosis, with identical cytoplasms; thus, cytoplasmic genetic variation between such ramets would not be a likely source of phenotypic variation among the pollen or progeny they produce (assuming enough pollen or progeny per ramet were sampled). Although a given ramet may produce pollen grains that vary with respect to the cytoplasmic genes received (if the microspore mother cell does not divide its cytoplasm equally among microspores during meiosis), pollen produced by vegetatively replicated ramets should not exhibit consistent between-ramet cytoplasmic differences (unless somatic mutations are common). To minimize potential variation between ramets resulting from pollen-borne cytoplasmic genes, we recommend that pollen donors representing a given genotype be replicated vegetatively, rather than by seed, when possible.

CONCLUSION

Environment-specific selection among developing male gametes or gametophytes should be recognized as a possible mechanism causing differences among pollen donors in the performance of their pollen or in the mean phenotype of their

progeny. We propose a breeding strategy-to be used when attempting to detect the presence of additive genetic variation in quantitative traits-that can control for or eliminate this type of gametophytic selection as a cause of paternal influences on pollen or progeny phenotype. The use of homozygous lines as pollen donors, when these lines are replicated within and across environments, can allow the detection of micro- and macroenvironmental paternal effects on progeny phenotype independently of environmentally induced meiotic drive. In addition, by replicating parental genotypes within and among environments, one can determine whether there are gender differences in the magnitude of micro- and macroenvironmental effects. The comparison of environmental effects on progeny produced by homozygous lines and heterozygous lines may also aid in the detection of environmentspecific selection among gametophytes. For populations with paternal environmental effects not attributable to gametophytic selection, reciprocal crosses among bisexual homozygous lines may be used to compare the strength of paternal, relative to maternal, environmental effects. The ability to resolve more highly the sources of and evolutionary significance of phenotypic variation in fitness-related traits may be improved by these comparisons.

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